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EDITED BY

SIMON FLEXNER, M.D.

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# DOES CARDIAC RHYTHM ALONE DETERMINE HUMAN BLOOD PRESSURE VARIATIONS?*

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PLATES 1 TO 6.

## INTRODUCTION.

The observation that blood pressure undergoes periodic variations with inspiration and expiration dates from the experiments of Stephen Hales (1) in 1733. Their nature and causes, though frequently reinvestigated, still remain subjects for physiological discussion. One phase of the question, the part played by variations in cardiac rate, is again considered in this investigation.

An extensive literature has grown about this question. Ludwig (2) observed these variations, placed the time of acceleration in the expiratory period, and disregarded its possible significance in the causation of pressure variations. It was held in similar disregard by Magendie (3), as well as by Donders (4). It remained for Ludwig's pupil Einbrodt (5) to show that in dogs the pressure rises in inspiration and falls in expiration. The increase in heart rate during inspiration, together with an increased blood flow through the thorax, was held to account for the inspiratory rise of pressure. Einbrodt, however, considered the first as of distinctly minor importance, for, after a rhythmic condition of the heart had been produced by vagus section, the blood pressure variations were frequently even increased.

Burden-Sanderson (6) confirmed the observation that the heart rate increased during inspiration. He believed that the enlargement of the thorax caused a belated expansion of the veins and the relaxing heart, which favored their filling. He reasoned that the more rapidly the cavities filled, the shorter the period of relaxation, the more vigorous its systole, and hence the higher the pressure.

Kowalewsky (7) and Zuntz (8) mention the variations of cardiac rate as contributing to the periodic blood pressure variations. François-Franck (9), Sommerbrodt (10), and Schweinburg (11) have made no reference to them in this connection.

Fredericq (12) demonstrated that probably these variations were dependent on a central influence and clearly analyzed the part they played in respiratory variations of blood pressure. He pointed out that variations of blood pressure occurred in the dog with respiratory phases, whether the heart was rhythmic or arhythmic. There was this difference: in the former case blood pressure fell

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during inspiration, in the latter it rose. He also pointed out that in animals where no normal variation in cardiac rate occurred (rabbit), the pressure always fell during inspiration. Fredericq, therefore, concluded that the mechanical influence of respiration tended to reduce arterial pressure during inspiration, but that in the dog these changes were overbalanced by an acceleration of the heart. Inasmuch as he observed a similar pulse variation in himself, he concluded that cardiac rhythm determined the direction of human blood pressure.

Various investigators, Riegel (13), Landois (14), Schreiber (15), Sommerbrodt (10), and others, using the sphygmograph as an indicator of blood pressure changes, concluded that, in man, a fall of pressure and not a rise occurred during inspiration. In 1886, however, Klemensiewicz (16) showed by simultaneous graphic records of respiration that the main change was a rise during inspiration which was preceded by a fall if cardiac acceleration was delayed in its onset. Werthheimer and Meyer (17) confirmed this and showed that atropin not only prevented the inspiratory cardiac acceleration but also the consequent rise in pressure. A glance at the published records of these investigators shows that only the lowest level of each pulse wave rises while the upper portion distinctly falls during inspiration. Such records tend to show that the cardiac rate determines the variation in diastolic pressure. Sphygmograph tracings taken from different individuals by other investigators, however, show both systolic and diastolic portions to vary as frequently in the same direction. While some of these variations may be attributed to individual variation, the point has been raised that the systolic and diastolic portions of sphygmograph records may not be regarded as a reliable index of the trend of pressure variations on account of the varying volume of the venæ comites (Hill, Barnard, and Sequeira (18), and Hirschman (19)).

More recently Lewis (20) attempted to obviate these objections against the use of the instrument as a pressure indicator by suspending it from the wrist. From seventeen experiments upon this point, he concluded that the most frequent type of pressure variation during normal breathing is due to heart rate variation, systolic and diastolic pressure changing in the opposite directions as the heart slows and accelerates. During deep thoracic or abdominal breathing, however, another form of variation is added which is due to the mechanical influence of respiration.

Last year Erlanger and Festerling (21) showed that "the size of the sphygmomanometer oscillations" (*i. e.*, diastolic pressure) "was not materially influenced by such pulse pressure changes as probably result from the changing systolic output," and further, that when breathing rate was voluntarily altered they followed the variations of respiration and not heart rate. A similar conclusion was arrived at by the mathematical analysis of Frank (22) who concluded that "moderate changes in the normal cardiac frequency can cause no change in blood pressure, since it already lies near the maximum." The writer (23) tested this question in the pulmonary circuit with the result that, within the range of cardiac cycle variation most commonly found in man (0.60 to 0.90), the length of the cardiac cycle was practically without influence on systolic and diastolic pressures as measured by an improved pulse pressure instrument.

This review establishes the idea that great variation in cardiac rate may determine entirely the trend of blood pressure changes.

It is generally admitted, however, that, in addition, there exists a mechanical influence due to respiratory movements which tends to exert a contrary influence on blood pressure and predominates when heart rate changes are not marked. It is not entirely agreed, however, whether the cardiac variations in man are great enough to exert any influence counteracting the effect of respiration itself, or whether there are individual variations.

Opposed to the current view as to the part played by respiration is that given in a recent communication by Henderson and Barringer (24). These investigators hold that it is impossible for the mechanical influence of respiration to modify arterial pressure except as it operates through a change in heart rate. Briefly stated their position is as follows: venous pressure in man and animals is always above a certain critical value, and therefore all beats of the mammalian heart are superimposable. This condition, however, is difficult to maintain in experimental animals and most experiments are performed with the venous pressure below the critical level. In such cases the most that respiration can do is to modify ventricular filling by periodically raising the venous pressure to a critical level, and arterial pressure may be modified independently of the rate. By special precautions (induction of hypercapnia and saline infusion), however, it is possible to preserve a critical pressure and reduplicate normal conditions. In such cases the systolic output and arterial pressure are determined solely by the length of the previous diastole. Henderson would harmonize the discordant observations as to whether the human pressure rises or falls in inspiration by saying that the change in cardiac rhythm bears no constant relation to these respiratory phases.

As this view rests upon experimental facts supporting the hypothesis that in normal animals it is physiologically impossible for mechanical influences of respiration to modify the blood pressure apart from changes in the heart rate, it becomes desirable to reinvestigate the question of how closely systolic and diastolic pressures in man vary with the duration of the previous heart cycles.

## THE ESTIMATION OF HUMAN PRESSURE VARIATIONS BY THE SPHYGMOSCOPE OSCILLATIONS.

### ANALYSIS OF PREVIOUS WORK.

The methods for detecting variation of human blood pressures have not been entirely satisfactory. They have been recently reviewed by Erlanger and Festerling (21) who, among other procedures, suggested a convenient method which may be briefly described as follows: The cuff of an Erlanger blood pressure apparatus was applied to an arm and the systolic pressure estimated. The pressure was then reduced five or ten millimeters, at which level a continuous record of pulsations, with the tambour attached to Erlanger's instrument, was recorded.

The method rests upon the principle that "if a pressure lying anywhere between systolic and diastolic be applied to an artery through a recording sphygmomanometer adapted to employ the principle of Marey, an oscillation is recorded whose amplitude is larger than that obtained at systolic pressure and smaller than that at diastolic pressure. If, while this pressure on the artery is maintained, . . . the intra-arterial minimum pressure should approach the extra-arterial pressure, the amplitude of the recorded waves should, as a result, increase; and *vice versa*, . . ."

It was, of course, evident to these investigators that the principle of Marey could be theoretically applied in this fashion to pressure measurements only provided that the pulse pressure were constant during consecutive beats of the heart, for upon the amplitude in pulse pressure the oscillations primarily depend. They, therefore, carried out animal experiments to demonstrate not only the validity of the theory that the amplitude of oscillations depended on the relation of extra-arterial to intra-arterial minimal pressure, but sought also to determine what modifying effect variations of pulse pressure exerted. Their conclusions on this point, here consecutively stated, were: (*a*) "Ordinarily, however, as experiments on animals demonstrate, pulse pressure changes do not mask blood pressure changes"; (*b*) "Nevertheless, one should keep in mind the danger of drawing conclusions from continuous blood pressure records without, at the same time, taking into consideration all of the factors at work in producing the changes";[1] and (*c*) in man, "the height of the oscillations is not materially influenced by such pulse pressure changes as probably result from the changing systolic output. . . ."

The experimental data, however, do not seem to establish satisfactorily the fact that these inferences may also be applied in cases where variation in the length of cardiac cycle is responsible for pulse pressure changes.

In the first place the records submitted show no satisfactory evidence that variations in diastolic pressure due to cardiac rhythm cause corresponding variations in the sphygmomanometer oscillations which may not as well be explained by pulse pressure variations or by the fact that the extra-arterial pressure was obliterating the vessel within the arteriograph. A case is pointed out by one of the authors (25) as bearing upon this point. For example, the systolic pressure

[1] Erlanger, J., and Festerling, E. G., *loc. cit.*, p. 381.

of the last vagus pulse of the second vagus stimulation in figure 3 in the article of Erlanger and Festerling rises almost to the same systolic level as the pulse following. The diastolic pressure of the former is far below that of the latter, yet despite the great difference in pulse pressure in favor of the vagus beat, the sphygmomanometer oscillations are distinctly smaller. To judge from the impact waves of the sphygmomanometer, however, the artery was entirely collapsed within the arteriograph during the vagus beat and not during the beat following, in which case no correspondence could be expected. Unquestionably, the lever throw during the vagus beat was greater than in the other beats recorded by the Hürthle manometer, so that the systolic pressure was actually less than that recorded in the vagus beats. This failure to control the fling of the manometer lever renders these records unsatisfactory evidence.

In the second place satisfactory evidence is not at hand to show that variations in pulse pressure due to heart rate changes do not mask blood pressure changes.

The fact[2] that the lower diastolic pressure and increased pulse pressure accompanying vagus stimulation shown in figure 3 is attended by smaller sphygmomanometer oscillations is seemingly conclusive that the change in diastolic pressure chiefly determines the amplitude in oscillation and that pulse pressure changes play an insignificant part. Another explanation is more plausible, however. With the fall of diastolic pressure the systolic pressure also falls and, to judge from the typical impact waves of the sphygmomanometer record, it falls so low that the extra-arterial pressure is always obliterating the artery and, in consequence, this amplitude is necessarily smaller. During the first vagus stimulation when the diastolic pressure is practically the same, only the first three waves, in which the systolic pressure is somewhat higher, gave anything that might be called a sphygmomanometer record. As soon as extra-arterial pressure is no longer above intra-arterial, as shown in the third vagus stimulation, we get, allowing for friction irregularities, a correspondence between oscillations and pulse pressure changes.

Inasmuch as a clear decision in the matter is imperative to determine the influence of cardiac rhythm on blood pressure changes, it was deemed desirable to reinvestigate these questions.

## EXPERIMENTS.

The sphygmoscope attachment of Erlanger's blood pressure apparatus was changed to the extent that a Frank's segment capsule, recording by a reflected band of light on a moving bromide film, was substituted for the tambour. By this change the quality of the apparatus was improved to such an extent that now, at least, the variations of pressure transmitted by the elastic ball of the sphyg-

[2] Erlanger, J., and Festerling, E. G., *loc. cit.*, p. 379.

moscope system were accurately picked up by the segment capsule and registered without friction on the writing surface. The sensitiveness was at the same time increased to such an extent that the pressure variations from the dog's femoral could be directly recorded. This was considered a distinct advantage, inasmuch as it is necessary in interpreting pressure measurements in man to deal with the elasticity coefficients of the overlying tissues and of the sphygmoscope system under different tensions and variable air contents. To this end, a conical cuff, containing a trapezoidal bag having a width of seven centimeters, was strapped to the thigh, and by special clamps so fastened to the tightly drawn skin that slipping was not possible. Preliminary tests showed that the pulsations in the artery peripheral to the bag, recorded by an exceedingly sensitive optical arrangement, were entirely prevented when the extra-arterial pressure was five to ten millimeters above the maximal pressure recorded from the other femoral by a maximum mercury manometer. In order to obviate the criticism, which according to the writer's opinion is unjust, that a considerably larger component of the pressure is lost in applying pressure to a flat and conical leg of a dog than by a cuff to the cylindrical arm of man, an arteriograph was applied to the carotid artery in three experiments. The variations in carotid pressure were recorded by Frank's optical manometer,[3] while the maximum and minimum pressure was read as from the other femoral artery. The normal variations in heart rate, the effects of natural breathing, of cutting vagi and stimulating either end, of compressing the trachea, and of atropin and adrenalin as these bore upon the relation between the two curves, were all investigated.

The results of eight such experiments were conclusive. They fail to show that where a method is employed similar to that used in man, variations of diastolic pressure due to changes in heart rate determine variations in the amplitude of oscillations of the sphygmoscope. Specific instances are shown where an extra-arterial pressure not more than ten millimeters below maximal in the other femoral was applied by the bag to the artery. In figure 1 the size of the

[3] In the later experiments the manometer was calibrated.

sphygmoscope oscillations 1 and 2 decreases when systolic pressure of the corresponding beat falls and the diastolic pressure remains unaltered (decreased pulse pressure). In figure 2 a progressive rise of diastolic pressure, due to an accelerated heart, causes not a larger but a smaller oscillation. In this case the highest maximal intra-arterial pressure reached was 167, the minimal was 75, and the extra-arterial was 150.

On the other hand, these records show that when variations in cardiac rhythm occur, an exact correspondence obtains between amplitude of oscillation and pulse pressure. Thus, in figure 1, when, during inspiration, the pulse pressure decreases, diastolic pressure remaining practically unchanged (waves 1 *a*, 1, and 2), the size of oscillations diminishes and what is of fundamental importance as showing the minimal determining influence of diastolic pressure, this relation held true for the entire range of extra-arterial pressures from above systolic to below diastolic. The three arteriograph experiments were particularly convincing upon this point. Records of experiments of which a few segments are shown in figure 3 illustrate this so clearly as to render detailed discussion superfluous. Experiments taken from the animal's leg with a cuff showed the same relations when the animal was in apnea, but, as the results of table I indicate, the movements incident to respiration in the dog occasionally impair an exact correspondence. The results are appended to show that even when extraneous influence sometimes impairs the results, the relation of the external to the diastolic pressure is always without effect in altering the relation between pulse pressure and sphygmomanometer oscillations. Inasmuch as a decrease in pulse pressure associated with a shorter cycle means a higher diastolic pressure, it follows that, contrary to the interpretation of Erlanger and Festerling, an increase in diastolic pressure in such cases is indicated by the smaller pulse pressure waves. It is necessary to lay stress upon this because the diastolic pressure changes will be misinterpreted when cardiac rhythm is not regular, if the size of the oscillations is taken as the criterion of diastolic pressure. This, of course, does not necessarily imply that larger oscillations may not accompany a rise of diastolic pressure. Figure 1, waves 3 and 4, show that, during natural breathing when

TABLE I.

*Experiment 13.*

| Observation. | Maximal intra-arterial pressure. | Extra-arterial pressure. | Minimal intra-arterial pressure. | *A.* Variation in diastolic pressure (Frank's manometer). *B.* Variation in pulse pressure (Frank's manometer). *C.* Variation in size of oscillations (sphygmomanometer). | |
|---|---|---|---|---|---|
| | | | | + − + + − + +[4] | *A* |
| 2 | 126 | 130 | 96 | − + − − + − + | *B* |
| | | | | − + − − o − + | *C* |
| | | | | + − + − + + | *A* |
| 3 | 128 | 152 | 98 | − + − + − o | *B* |
| | | | | +? −? − + − + | *C* |
| | | | | − + − − + − + | *A* |
| 4 | 118 | 118 | 92 | + − + o − + − | *B* |
| | | | | + − + − + −? −? | *C* |
| | | | | − − − + + − − | *A* |
| 5 | 118 | 100 | 90 | + o + − − + + | *B* |
| | | | | + o + − − + + | *C* |
| | | | | + − − o + − − | *A* |
| 6 | 118 | 90 | 88 | − + o o − o + | *B* |
| | | | | − + + − o o + | *C* |
| | | | | o − + − − − + | *A* |
| 7 | ? | 80 | 88 | o o − o + o − | *B* |
| | | | | − + − + + + − | *C* |
| | | | | − o − − − − + + | *A* |
| 8 | 138 | 70 | 90 | + o o o o o − − | *B* |
| | | | | ? − − + o o − − | *C* |
| | | | | o − + + − − + | *A* |
| 9 | 160 | 85 | 88 | o + − o + o − | *B* |
| | | | | o + − − + + − | *C* |
| | | | | − o + − o − | *A* |
| 10 | 160 | 160 | 88 | o o − + o o | *B* |
| | | | | o − − + o o | *C* |

the heart is regular, large oscillations often accompany an increase in diastolic pressure, but the greater coincident pulse pressures may account for the changes. Nor do these experiments necessarily disprove the possibility that the amplitude of oscillation may be determined by the relation of intra-arterial diastolic pressure to extra-arterial pressure when heart rate and pulse pressure remain unaltered. Inasmuch as such conditions are rarely approximated in man, however, they scarcely permit the application of the criterion to establish human pressure variations.

[4] + = an increase; − = a decrease over the preceding wave; o = no change.

Since variations in amplitude of oscillations parallel changes in the pulse pressure, it became desirable to investigate whether the troughs and crests of the sphygmoscope oscillations registered by photographic means follow variations in systolic and diastolic pressure of Frank's manometer. If the volume changes of an artery induced by a pressure variation within the vessel could be transmitted to an ideal manometer system externally applied and sustaining a pressure less than systolic, they would induce similar but much smaller changes in this manometer. If these could be recorded without error, they would be proportionate to the variations of intra-arterial pressure. Hürthle (26) attempted to apply this principle in exact estimation of human pressures. Although Frank (27) has pointed out that this can not be realized with such a method, it may still be conceived that an instrument of much lower quality may show a directional but not a quantitative change in pressure.

A comparative study of animal experiments shows that, during apnea, *c. g.,* after curare, during central vagus stimulation, and after adrenalin, an exact qualitative but by no means a quantitative correspondence exists. The troughs and crests, beat by beat, change in direction with the diastolic and systolic pressures of Frank's manometer (figure 2). This correspondence holds not only when the extra-arterial pressure is equal to the diastolic within the artery but is equally faithful up to the systolic level.

A study of the arteriograph records, during which animals were breathing, showed also a general correspondence in the variations of the sphygmoscope record and Frank's manometer (figure 3). Many isolated exceptions were present, however, in the records taken from the leg which it is necessary to analyze in order to recognize the limitations of the method in man. Thus in figure 1, to judge from waves 1 and 2 of the sphygmomanometer record, both systolic and diastolic pressures are falling. A glance at the corresponding pressure waves shows, however, that systolic pressure alone fell in wave 1 and rose again in wave 2. Again, wave 4 gives the impression that an increase in both systolic and diastolic pressure over the preceding beat occurred, whereas the direct pressure record shows that systolic pressure decreased and diastolic pressure remained unchanged.

An analysis of the cases where discrepancies occurred showed that they could be referred to extraneous movements imparted by respiratory or voluntary muscular contractions of the limb to the bag. The latter are easily recognizable on the curves from animals and man (figure 4), and when present the records may be discarded. The respiratory variations are not always so clearly shown (figure 1), and in the dog they are exceedingly difficult to obviate since every respiration exerts a tug upon the skin of the thigh throughout the inguinal fold of skin connecting the abdomen with the thigh. It follows that the troughs and crests of individual sphygmomanometer variations follow the direction of systolic and diastolic pressures within the artery accurately only when extraneous influence can be guarded against. The experimental plan of the method, therefore, shifts to man.

Fortunately, on account of anatomical differences, these movements can probably be controlled with attention to a proper placement of the arm. If an arm bag is applied to the arm hanging to the side, one may obtain marked variations of the whole curve with respiratory movements which disappear entirely or become altered in direction when the arm is allowed to rest comfortably in a horizontal position away from the chest. In control experiments no variations that could be attributed to such an influence were discernible when a second narrow cuff with a pulse-obliterating pressure was placed above the recording cuff.

It may be concluded then, that, during natural breathing, when precautions are taken that no extraneous movement is transmitted to the cuff, the crests and troughs recorded by an air-tight sphygmoscope system give respectively a directional indication of systolic and diastolic pressures occurring in man.

### THE RELATION BETWEEN SYSTOLIC AND DIASTOLIC PRESSURES AND THE LENGTHS OF THE CARDIAC CYCLES IN MAN.

*Method of Experimentation.*—The subjects reclined upon a bed, their left arms, strapped with a blood pressure cuff, resting away from the thorax. Each subject unacquainted with the objects of the experiment was allowed to rest quietly for several minutes. The systolic pressure was then roughly determined by palpating for

the return of the radial pulsation and the instrument set so that the pressure was 10 to 15 millimeters below this level. The oscillations of the sphygmoscope transmitted by an air-tight system to a Frank's segment capsule, together with the movements of a respiratory tambour communicating with a pneumograph and a tuning fork with a vibrating period of 0.02 of a second, were then projected upon a moving bromide film.

As a rule, the breathing of these quietly resting subjects was entirely involuntary and shallow. When a record had been obtained under these conditions the subject was told to breathe perfectly naturally. The psychic element thus introduced resulted, without fail, in deepening the breathing. This respiration, however, did not exceed in depth that of the normal individual. It is designated as voluntary respiration to distinguish it from the involuntary type naturally assumed. In a number of cases the effects of deep breathing and apnea were also recorded.

*Method of Analyzing the Results.*—The developed records obtained from thirty-five different subjects were analyzed as follows: The cardiac waves were consecutively numbered and their relation to inspiration and expiration was determined. The duration of each cardiac cycle was next expressed in hundredths of a second. By the aid of the millimeter scale photographed on the film, the relative relations of the troughs and crests of the waves were directly determined and expressed in terms of millimeters (figure 5).

These figures, which, according to previous discussion, could give only a criterion of qualitative changes in systolic and diastolic pressures occurring during consecutive beats, were plotted on the ordinates of cross-section paper.[5] To facilitate plotting, the beats were laid off as equal lengths on the abscissæ, the actual duration of the cardiac cycle being numerically indicated for each wave. In this way it was possible to study whether the variations in diastolic and systolic pressures of consecutive waves[6] accorded with those ex-

[5] As the relative changes in systolic and diastolic pressures were alone of importance the actual figures have been omitted from the plot.

[6] The diastolic pressure for any wave (*e. g.*, wave 1, figure 1) is considered throughout the paper as the lowest pressure reached previous to the systole of that beat (point a), and the term systolic pressure is applied to the highest pressure reached during the systole of that beat (point b).

pected, provided the length of the preceding cardiac cycles solely determined these pressures. In order to do this it is essential to bear constantly in mind the anticipated effects of any change in heart cycle.

It is generally recognized that a lengthening of the cardiac cycle increases, and a shortening decreases the mean pressure of the succeeding beat. This, however, only imperfectly relates the pressure effects, since the inference might be drawn that both systolic and diastolic pressures necessarily follow the directional change of the mean pressure, which is not always the case in animals. When, for example, variations in cardiac rate solely determine the blood pressure changes in anesthetized animals in apnea, as shown by Frank's mirror manometer (N = 170), it is the rule that shortening of a cardiac cycle increases the diastolic and decreases the systolic pressure of the beat following (figure 2, *a* and *b*). The same changes occur in naturally breathing animals when cardiac rhythm controls the pressure (figure 7). The systolic pressure deviates from this rule, however, (*a*) when peripheral constriction is great (after adrenalin or asphyxia), (*b*) when the shortening of the cycle is not sufficient to alter the degree of filling (in very slow heart beats), and (*c*) when a progressive acceleration lasts for a considerable period so that the systolic pressure is supported by the diastolic (figure 3, *c, d, e*). Conversely, a lengthening of the cardiac cycle causes an elevation of the systolic and a fall of the diastolic pressure of the following beat. Here also the systolic pressure may fall under conditions opposite to that outlined above. In all cases, however, the pulse pressure decreases when the heart accelerates, and increases when it slows.

In dogs with rhythmic hearts, or where respiration controls the pressure variations, inspiration causes a fall of both systolic and diastolic pressures, and expiration a rise (figure 1). The pulse pressure increases during expiration and decreases during inspiration. Systolic and diastolic pressures change in the same direction.

*Results.*—To facilitate an analysis the experiments were grouped in accordance with the points they illustrated, and from these groups a number of representative plots are briefly discussed (figure 8).

INFLUENCE OF HEART CYCLES DURING APNEA.

*Experiment 32.*—Breath held in expiration. Very slight variations (0.01 of a second) in rhythm occur and the systolic and diastolic pressure of consecutive beats do not vary.

*Experiment 35.*—Voluntary apnea. Heart cycles increase in length slightly (0.02 of a second). This is without influence on systolic pressure, but a slight decrease in diastolic pressure is evident. At the fifth beat, *i. e.*, after a series of progressively lengthening beats, the systolic pressure falls, due to lack of diastolic support.

*Experiment 33.*—Voluntary apnea with very slow heart rate. A lengthening of the third cycle plotted causes a fall of diastolic pressure and a decrease in the systolic pressure of the fourth wave. A shortening of the fourth cycle in the fifth wave increases both diastolic and systolic pressures.

*Experiment 36.*—Voluntary apnea. Average cardiac cycles. A progressive decrease of the cardiac cycles during the first five waves results in a progressive rise of diastolic and a fall of systolic pressures. At the sixth and seventh waves, the systolic pressure rises, due to increased diastolic support resulting from a continued decrease in length of heart cycles. As the cycle increases again in the seventh wave, the diastolic pressure decreases, but systolic does not rise for a beat, probably because the systolic pressure was already high due to diastolic support.

*Experiment 37.*—Voluntary apnea. A progressive and pronounced lengthening of the heart cycle takes place. The progressive decrease of diastolic pressure is accompanied by a systolic rise in the first three waves. The systolic pressure does not continue to rise on account of the greater diastolic drop and, for the same reason, it falls in the sixth and seventh waves.

These experiments, which were the only ones in a series of twenty apnea experiments in which marked cardiac variation occurred during apnea, show that when respiration is in abeyance, variations of cardiac rhythm, except when very small, modify the pressures of man in precisely the same manner as was above stated to be the case in animals.

From this the rule may be formulated that the duration of the preceding cycle probably cannot be considered to govern the pressures unless it can be shown that:

(1) a decrease in systolic pressure is (*a*) preceded by a cycle shorter than that of the wave before (*e. g.*, waves 4 and 5, experiment 36), and accompanied by an increase of diastolic pressure, or (*b*) preceded by a cycle of the same or a longer length than that of the wave before, and by a decrease of diastolic pressure for several previous beats (*e. g.*, waves 5, 6, and 7, experiment 37); and,

(2) an increase in systolic pressure is (*a*) preceded by a cycle

longer than that of the wave before and accompanied by a decreased diastolic pressure (*e. g.*, waves 2 and 3, experiment 37), (*b*) preceded by a cycle of the same or a shorter length than that of the wave before, and by an increase of diastolic pressure for several previous beats (*e. g.*, waves 6 and 7, experiment 36).

INFLUENCE OF RESPIRATION WITHOUT CHANGES IN CARDIAC RHYTHM.

*Experiment 21.*—Involuntary breathing, moderate depth. After the first wave plotted the cardiac cycles are equal. In spite of this fact systolic pressure falls distinctly during inspiration.

*Experiment 17.*—Involuntary breathing, moderate depth. The duration of the cardiac cycles determining the first five beats is practically uniform. In spite of this the systolic pressure undergoes a fall during inspiration and a rise during expiration which can only be accounted for as an influence of respiratory movement on blood pressure.

*Experiment 24. Curve A.*—Involuntary breathing, moderate depth. Although the heart cycles are equal, systolic pressure is somewhat lower during inspiration and higher during expiration. *Curve B.*—Voluntary, deeper breathing. The inspiratory phase is prolonged and the fall of both systolic and diastolic pressures during this phase is more prominent. Since the cardiac cycles determining waves 2 and 3 and also 4 and 5 are of the same length, the pressure change may be attributed to the effect of inspiration. The pressure changes of wave 6, on the other hand, might be attributed to a cardiac influence.

The experiments, of which these are illustrations, show that, in cases where cardiac variations are negligible or absent, mechanical respiratory influences lower the systolic pressure when breathing is shallow, and the diastolic pressure also when breathing becomes somewhat deeper. As in animals, systolic and diastolic pressures vary in the same direction.

INFLUENCE OF SLIGHT VARIATIONS IN CARDIAC RHYTHM DURING NATURAL RESPIRATION.

*Experiment 35. Curve A.*—Shallow, involuntary breathing. A fall of systolic pressure during inspiration occurs. Cardiac rhythm is not responsible for the change because the fall of systolic, with a slight tendency to a rise of diastolic pressure, follows a consecutive lengthening of the cardiac cycles; and although a variation in the duration of the cardiac cycle occurs during the two beats of inspiration, the pressure remained unaltered for these beats. *Curve B.*—Deeper voluntary breathing. Waves 2, 3, 4, and 5 might be accounted for by cardiac rhythm, but the systolic decrease of the sixth wave could scarcely be attributed to the lengthened cycle preceding it, nor could the fall of diastolic pressure for the seventh wave be accounted for by the shorter cycle of wave 6.

*Experiment 22. Curve A.*—Shallow, quiet breathing. A very slight reduction of systolic pressure coincident with inspiration occurs. The slight variations

of waves 1, 2, 3, and 4 during expiration are easily explained by heart rate variations. The fall of systolic pressure of wave 5, though slight, cannot have been due to such variation. *Curve B.*—Voluntary breathing. The sudden increase in systolic pressure of waves 3 and 4 could not possibly be attributed to heart rate variations. Nor could the fall of systolic and diastolic pressures during waves 7 and 8 be referred to the shortened cycles preceding them.

*Experiment 19. Curve A.*—Shallow, involuntary breathing. The shortest cardiac cycles do not appear during inspiration and the cardiac cycles determining the first to the fourth waves are practically of the same duration. Still the systolic pressure falls during inspiration. The short cycles occur in expiration where wave 5 is followed by an unexpectedly high systolic pressure with no corresponding increase in diastolic pressure. Upon increasing the depth of respiration (curve *B*), the systolic and diastolic pressures follow the respiratory phases exactly. The determining cycles for waves 2 and 3 do not vary greatly, yet systolic and diastolic pressures both fall. Waves 4 and 5, determined by the same cycles, show a pronounced difference in pressure not to be accounted for by rate variation.

*Experiment 32.*—Finally, in figure 5, is shown a sample record of the pressure variations obtained during shallow breathing. During inspiration, which begins after the second wave, a fall of systolic pressure takes place. During expiration, which begins after the third beat, the systolic and diastolic pressures both increase. The fall in systolic pressure of the third wave is probably not the effect of a shortening of the second heart cycle, for a similar shortening in expiration does not reduplicate the directional change in the fifth beat. The rise of diastolic pressure of the fourth beat is also difficult to refer back to a lengthened cycle in wave 3.

Although moderate changes in cardiac rhythm may play a part in modifying systolic and diastolic pressures, the influence of respiration is paramount, for, no matter what relation exists between cardiac and respiratory rhythm, the systolic pressure, at least, always falls during inspiration and rises during expiration in these cases.

INFLUENCE OF THE AVERAGE RHYTHM VARIATION (MODERATE) DURING NATURAL RESPIRATION.

*Experiment 25.*—Quiet, involuntary breathing (figure 6). The systolic and diastolic pressures of waves 2 to 5 may be explained as due to cardiac variation, although it cannot be denied that inspiration may play a part in determining waves 4 and 5. Wave 6, following a shorter cycle, shows a higher systolic pressure without previous elevation of the diastolic to account for the systolic rise on a summation basis. On the other hand, it is significant that the deviation occurs at the beginning of expiration.

*Experiment 34.*—Quiet, involuntary breathing. The systolic pressures of waves 2 and 3 during inspiration are equal yet determined by different length cycles. The cycles governing waves 4 and 5 in expiration are equal, but both systolic and diastolic pressures increase in the latter beat.

*Experiment 30. Curve A.*—Quiet, involuntary breathing. Every pressure

change can be explained by a variation of cardiac rhythm. That respiration has a modifying influence, however, is suggested by the fact that, although the systolic and diastolic pressures of waves 5 and 6 both rise, the pulse pressure is greater. *Curve B.*—When breathing becomes deeper, respiration alters the direction of the pressures, as determined by heart rate variation. The lower systolic pressure of waves 4 and 5 and the higher systolic pressure of wave 6 are directly opposite to those expected if determined by previous heart cycles. The diastolic pressure also fails to agree with heart rate variation.

These cases show that when a considerable variation in cardiac rhythm occurs, especially when cardiac acceleration is limited to inspiration, a casual glance leaves the impression that systolic and diastolic pressures are entirely governed by the previous cardiac cycle. A careful perusal, however, shows that in each case one or more beats occur during every shallow respiration that cannot be accounted for upon this basis, and, in many cases, when breathing becomes deeper the effect of respiration becomes dominant.

MARKED RESPIRATORY ARHYTHMIA.

*Experiment 29 A.*—Moderately deep, involuntary respiration. Variations in cardiac cycle (0.23 of a second) entirely accounted for many waves not plotted. Wave 2, during inspiration, is governed by the same cycle as wave 1 in expiration, but the latter has a much reduced systolic pressure. The reduced systolic and increased diastolic pressure of the other waves in inspiration may, however, be accounted for by cardiac variations. Waves 5, 6, and 7, having practically cycles of the same length, show an increase of both systolic and diastolic pressure during expiration. It is interesting to note that wave 2, governed by a heart cycle of 0.83 in inspiration, is no larger than wave 7 governed by a cycle of 0.61 in subsequent expiration.

*Experiment 16.*—Moderately deep, voluntary breathing, cardiac cycle variation 0.23 of a second. With a progressive decrease of the cardiac cycle we find a decrease in systolic and an increase in diastolic pressure. The low diastolic pressure of wave 6 and the higher systolic pressure of wave 7 are difficult to accredit to the shorter cycles preceding.

*Experiment 13.*—Involuntary, moderate breathing, cardiac cycle variation 0.30 of a second. The variations of both systolic and diastolic pressures correspond to cardiac variations entirely. Wave 3, following a longer cycle, shows the characteristic lower systolic pressure which is the rule in slow hearts. A respiratory influence may exist but its invocation to explain the changes is not necessary.

*Experiment 7.*—Moderately deep respiration, cardiac variation 0.29 of a second. Systolic and diastolic pressure can be accounted for by heart rate variation. The rise of diastolic and fall of systolic with consecutive shortening until after the fifth wave leads to an increase of systolic pressure due to summation, is typical of cardiac rate changes. No necessity exists for the assumption of intervention by respiration.

*Experiment 18.*—Dog trained for calorimeter work. Figure 7 shows the

characteristic pressure variations of the normal, unanesthetized dog. Such pronounced variations in rhythm are never encountered in man. With inspiratory acceleration the diastolic pressure rises and systolic pressure falls until after several beats the summated effect causes the systolic pressure also to rise slightly. No evidence is found that mechanical movements of respiration play a part.

It may be concluded that in cases where extreme variations in cardiac rhythm occur these variations often obscure entirely any effect that movements of respiration may have on the systolic and diastolic pressures.

## CONCLUSIONS.

Changes in cardiac rhythm are not the only determinants of blood pressure variations in man. They play a part in the variations of systolic and diastolic pressures, the relative importance of which depends on the degree of arhythmia present and on the depth of respiration which, in itself, causes the systolic and diastolic pressures to decrease during inspiration and increase during expiration. No degree of rate variation can be regarded as a type. The cases range from those in which respiration governs the change of pressures entirely through those where more or less complicated mixtures of heart rate and respiratory influences intermingle to those in which extreme cardiac variations alone determine the pressure changes.

The emphatic insistence of Henderson and his collaborators that heart rate changes play a more important part than is commonly recognized in man is true in many cases. That, in certain cases, it is the only determining influence may also be admitted, but they are in the minority. The majority show the intervention of a respiratory influence which controls, at least, the variations of systolic pressure. In the light of these results, the doctrine that, in man, an effective venous pressure exists sufficient during all respiratory phases to insure superimposable beats, must be subjected to further reinvestigation.[7]

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## EXPLANATION OF PLATES.

### Plate 1.

Fig. 1. Natural respiration. The amplitude of the sphygmoscope oscillations corresponds to pulse pressure changes and not to variations in diastolic pressure. Troughs and crests fail to follow intra-arterial systolic and diastolic pressures if breathing movements are communicated to limb. R = respiration, inspiration upstroke; P = carotid pressure recorded by Frank's optical manometer; S = sphygmoscope oscillation. The numbers are referred to in the text. About three fourths actual size.

Fig. 2. Apnea. Close correspondence of troughs and crests of sphygmoscope record with systolic and diastolic changes recorded by Frank's manometer. The lettering is the same as in figure 1. About two thirds actual size.

### Plate 2.

Fig. 3. Five segments of records, showing that variations of pulse pressure occasioned by rhythmic cardiac changes determine the amplitude of the sphygmoscope oscillations obtained from an arteriograph. In the upper curve alone, where the intra-arterial systolic pressure fell below extra-arterial, and conse-

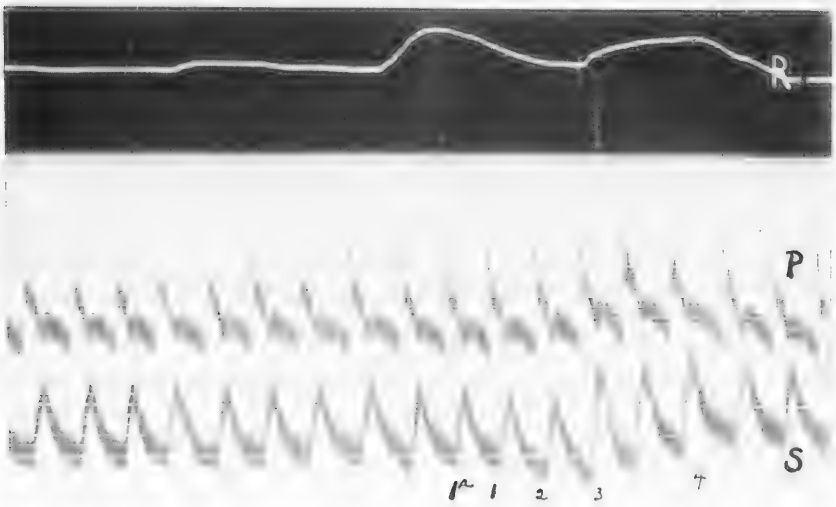


FIG. 1.

FIG. 2.

(Wiggers: Cardiac Rhythm.)

Mau

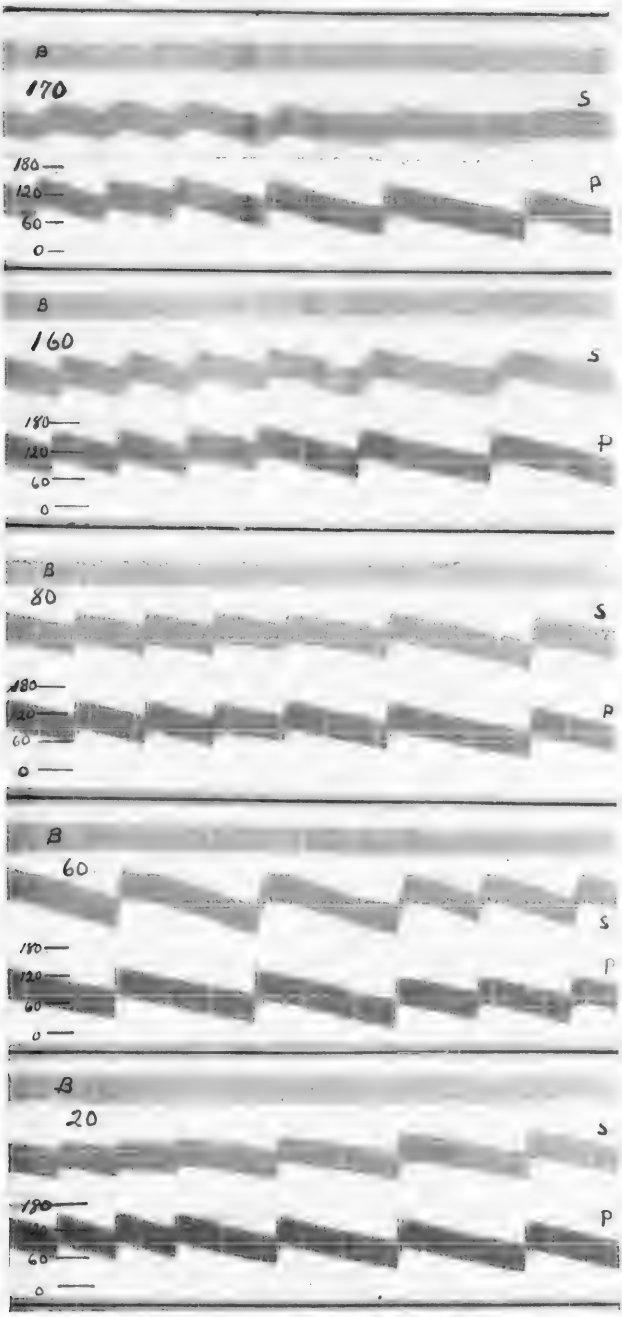


FIG. 3.

(Wiggers: Cardiac Rhythm.)

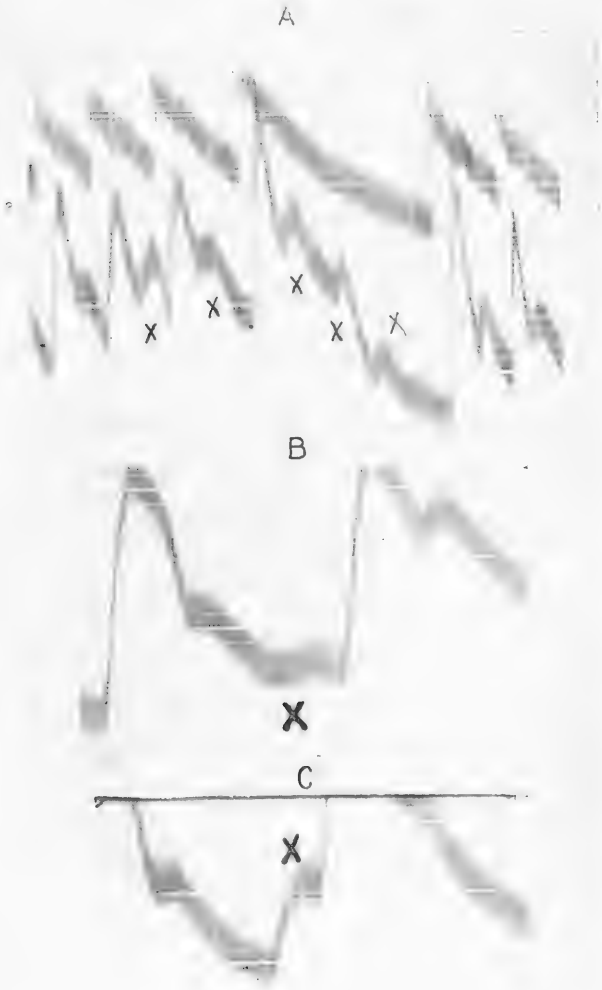


FIG. 4.

(Wiggers: Cardiac Rhythm.)

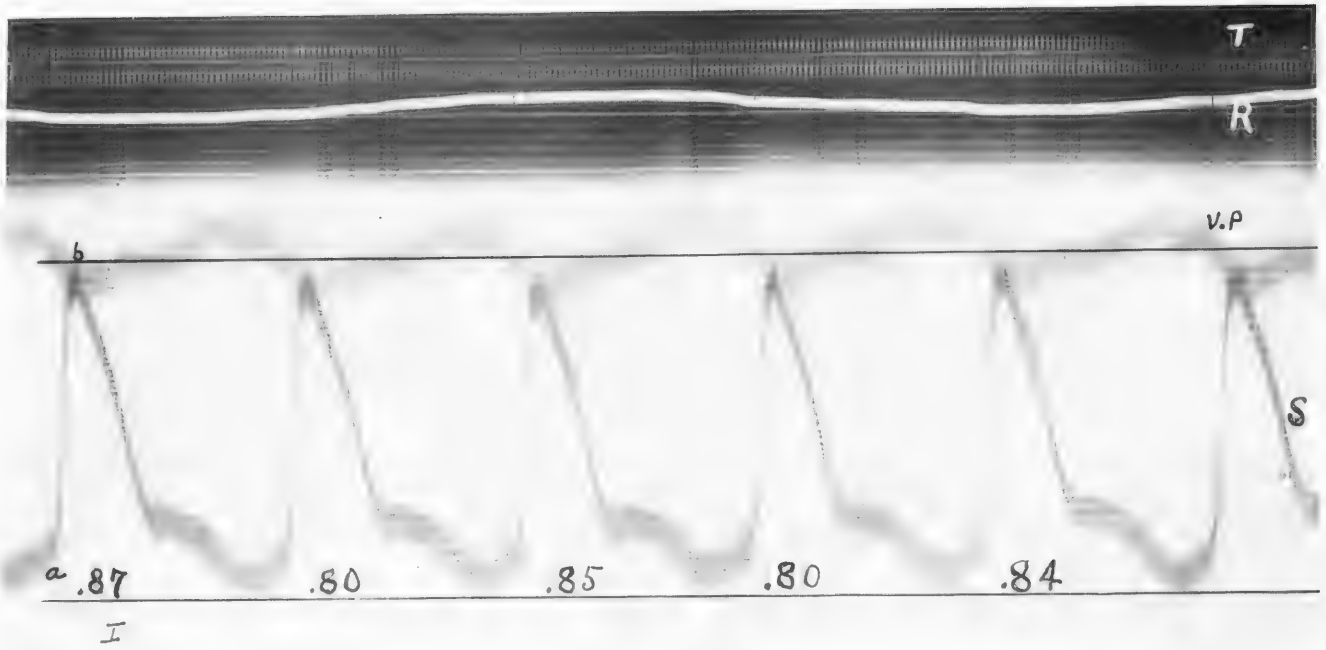


FIG. 5.

(Wiggers: Cardiac Rhythm.)

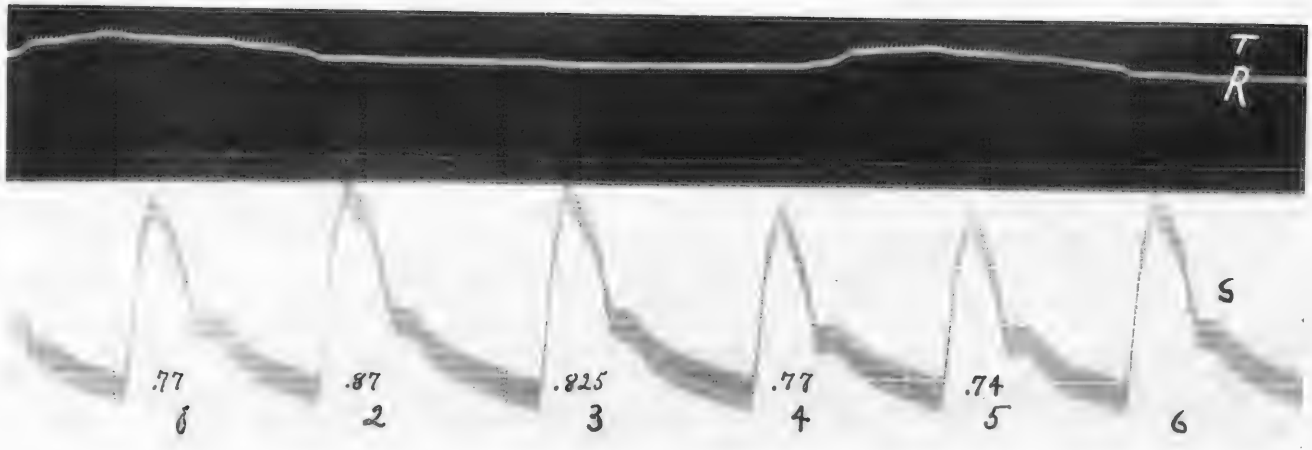


FIG. 6.

FIG. 7.

(Wiggers: Cardiac Rhythm.)

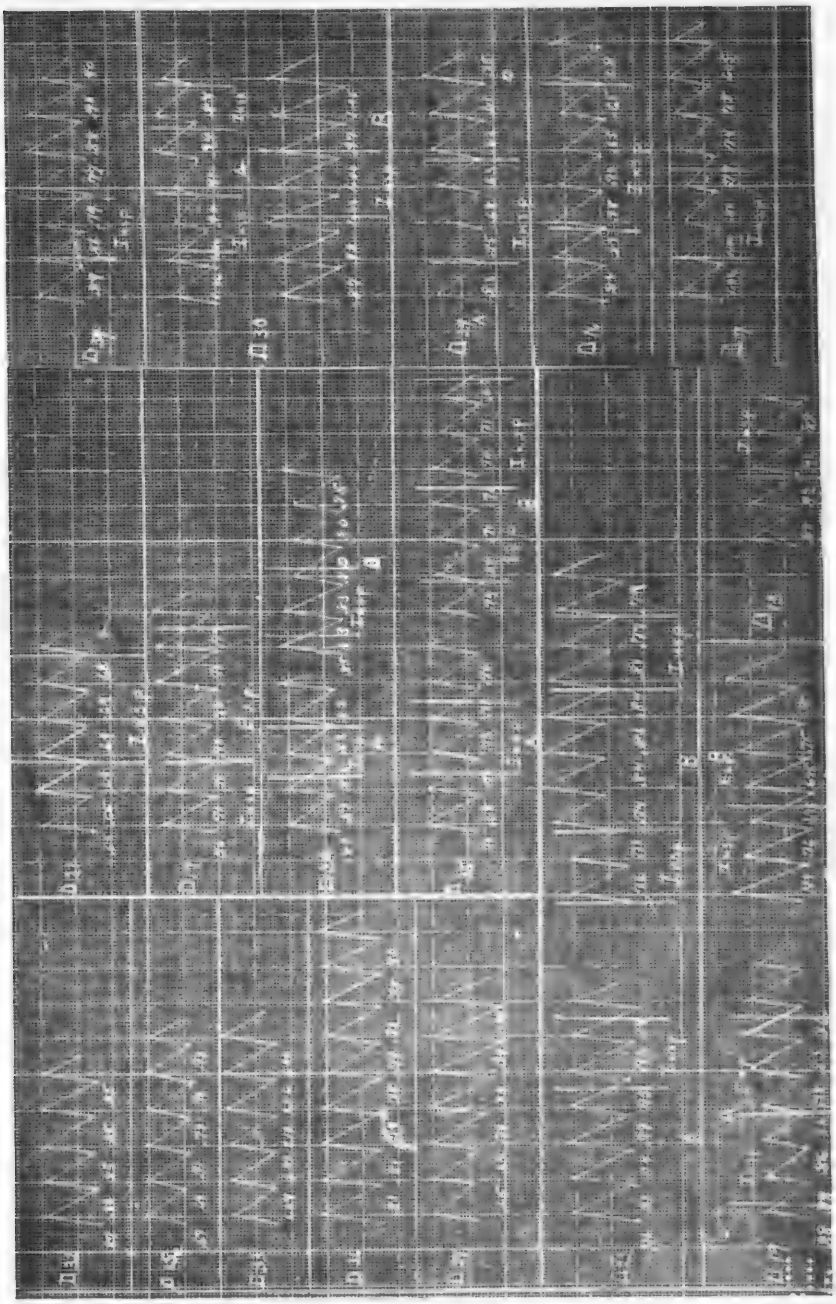

FIG. 8.

Wiggers: Cardiac Rhythm.)

quently obliterated the vessel, does a fall of diastolic pressure together with an increase of pulse pressure result in smaller oscillations. The lettering is the same as above. B = the base line from the top of which calibration was made, hence the calibration scale on Frank's pressure record (P) applies to the top of the line. About two thirds actual size.

PLATE 3.

FIG. 4. Three segments of records showing at X the effect of extraneous muscle contractions, (A) in the dog, and (B and C) in man. Actual size.

PLATE 4.

FIG. 5. Human pressure variations, involuntary shallow breathing, slight heart rate changes. T = time in 0.02 of a second. R = respiration, inspiration upstroke. S = sphygmoscope oscillations. V. P. = jugular pulse, of no significance in present connection. About five sixths actual size.

PLATE 5.

FIG. 6. Human pressure variations, involving quiet breathing. Average rhythm variation. The lettering is the same as before. About five sixths actual size.

FIG. 7. Pressure variations of an unanesthetized dog resting and breathing quietly. Marked cardiac arhythmia. About actual size.

PLATE 6.

FIG. 8. Plots from experiments showing the relation of systolic and diastolic pressures to heart cycles and respiration.

# OBSERVATIONS ON THE GROWTH OF BACTERIA ON MEDIA CONTAINING VARIOUS ANILIN DYES.*

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In recent communications Churchman (1) has described a selective action on bacteria of gentian violet. Stated in general terms, he found that bacteria were divided into two groups, of which one fails to develop in media containing gentian violet, while the other grows abundantly in the presence of this dye. The former group corresponds roughly with the Gram-positive group, the latter with the Gram-negative group of bacteria. His conclusions are based mainly on results obtained with dilutions of 1 to 100,000 of the dye.

A striking variation was noted in some enteriditis strains. These strains were identical by all ordinary cultural tests; one strain, however, persistently refused to grow on gentian violet agar. A subsequent examination showed that this strain could be differentiated from the others by agglutination.

Our interest in the use of media containing dye stuffs was further aroused by an article by Signorelli (2). He stated that on agar containing dahlia, cholera vibrios develop colored colonies by absorption of the dye with decolorization of the medium, whereas non-cholera vibrios give colorless colonies. We (3) were unable to verify these findings. In the course of the work we found two non-cholera vibrios which uniformly refused to grow on agar containing dilutions of dahlia which had no influence whatever on the growth of similar vibrios. Because of the above results, a few preliminary observations with various anilin dyes were made, which showed differences among related bacteria, and also differences in the reaction of the same bacteria to different dilutions of the same dye. The results of the comparison of the action of various dyes are given in this paper.

For the tests, the common pathogenic bacteria which grow freely

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on ordinary agar were selected. *Bacillus subtilis* was included as it is very sensitive to the presence of gentian violet. Other bacteria requiring serum or other similar additions were tried in a limited way, but this was discontinued as the albumins interfered with the activity of the dye.

As a routine a batch of agar was selected which was found especially suitable for the more feebly growing types. To the hot agar an appropriate volume of a watery solution of dye was added to give the final dilution desired. The same agar was used in each experiment.

The most convenient and economical method was found to be as follows. One unopened Petri dish was used to tilt up one side of a second dish. In the opposite side was poured just sufficient agar to give a satisfactory slant. This dish was covered and used to tilt up a third dish, and so on in a row. After the agar had set, slants were poured in the other side of the Petri dishes which were tilted in the reverse direction. In this way two mixtures of agar could be used in the same dish, very little agar being required for each slant.

In the general observations that follow two dishes were used containing the control agar, and agar with 1 to 500,000, 1 to 100,000, and 1 to 50,000 dilutions of the dye. The weakest dilution was used in the plate with the control.

For inoculation a fresh broth culture was usually employed. A small loop was used and a streak made on the agar slant, a fresh loopful being taken for each slant. Very heavy seeding as from agar slants tends to give irregular results, probably because of lack of close contact between the bacteria and dyed agar.

Table I gives the results of a series of tests after eighteen to twenty-four hours' incubation.

Some allied bacteria showed variations as noted in the table; namely, the streptococcus-pneumococcus group, the dysentery group, the capsulatus group, and diphtheria and the morphologically allied types. More complete tests of these groups were made.

Naphthylamin blue R gave an apparent difference between diphtheria and diphtheroid bacilli. Thirty-four strains of these bacilli were, therefore, planted on agar containing this dye. There was

TABLE I.

| Type. | Bacteria tested. | Hoffman violet. 1:50,000 | Hoffman violet. 1:100,000 | Hoffman violet. 1:500,000 | Crystal violet. 1:50,000 | Crystal violet. 1:100,000 | Crystal violet. 1:500,000 | Dahlia. 1:50,000 | Dahlia. 1:100,000 | Dahlia. 1:500,000 | Fuchsin. 1:50,000 | Fuchsin. 1:100,000 | Fuchsin. 1:500,000 | Rosanalin violet. 1:50,000 | Rosanalin violet. 1:100,000 | Rosanalin violet. 1:500,000 | Cresylechtviolett. 1:50,000 | Cresylechtviolett. 1:100,000 | Cresylechtviolett. 1:500,000 | Anilin violet. Gentian B. 1:50,000 | Anilin violet. Gentian B. 1:100,000 | Anilin violet. Gentian B. 1:500,000 | Naphthylamin blue R. 1:50,000 | Naphthylamin blue R. 1:100,000 | Naphthylamin blue R. 1:500,000 | Bleu de Lyon. 1:50,000 | Bleu de Lyon. 1:100,000 | Bleu de Lyon. 1:500,000 | Säureviolett (Kühne). 1:50,000 | Säureviolett (Kühne). 1:100,000 | Säureviolett (Kühne). 1:500,000 | Methylviolet 6 B. 1:50,000 | Methylviolet 6 B. 1:100,000 | Methylviolet 6 B. 1:500,000 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram-positive | Staphylococcus aureus | − | − | − | − | − | − | − | − | − | − | − | + | − | − | −* | + | + | + | − | − | − | + | + | + | + | + | + | + | + | + | − | − | − |
| | Streptococcus (green) | − | − | + | − | × | + | −* | × | ± | × | ± | + | − | ± | + | + | + | + | − | × | + | + | + | + | + | + | + | + | + | − | ± | + |
| | Streptococcus (hemolytic) | − | − | ± | − | − | ± | − | − | + | − | −* | + | − | − | ± | ± | ± | + | − | − | + | + | + | + | + | + | + | + | + | − | − | + |
| | Streptococcus mucosus capsulatus | − | − | + | − | − | ± | . | . | . | − | × | + | − | − | + | ± | ± | + | − | − | + | + | + | + | + | + | + | + | + | − | − | + |
| | Pneumococcus | − | − | ± | − | − | + | − | −* | + | − | ± | + | − | − | + | − | − | + | − | × | + | + | + | + | + | + | + | + | + | − | * | + |
| | B. diphtheriæ | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | × | − | − | − | ± | ± | + | + | + | + | + | + | − | − | − |
| | Diphtheroid bacilli (Cameron) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | + | + | + | + | + | − | − | − |
| | Diphtheroid bacilli (Cunningham) | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | . | . | . | − | − | − | − | − | + | + | + | + | + | + | − | − | − |
| | B. hoffmanni | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | ± | − | − | − | − | ± | + | + | + | + | + | + | − | − | − |
| | B. subtilis | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | + | + | + | − | − | − | + | + | + | + | + | + | + | + | − | − | − |
| | B. lactimorbi | − | − | − | − | − | − | − | − | + | − | − | ± | × | ± | + | − | − | − | + | + | + | + | + | + | + | + | + | + | − | − | * |
| | B. anthracis | − | − | − | − | − | − | − | − | −* | + | − | − | − | + | + | + | − | − | + | + | + | + | + | + | + | + | + | + | − | − | − |
| Gram-negative | Cholera | − | − | + | − | − | + | − | × | + | + | + | + | − | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | × | + |
| | Vibrio (indol-positive) | − | −* | + | − | − | + | × | ± | + | + | + | + | − | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | × | + |
| | Vibrio (indol-negative) | − | − | + | − | −* | + | × | ± | + | + | + | + | − | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | * | + | + |
| | B. proteus | ± | + | + | −* | × | + | ± | + | + | + | + | + | + | ± | + | + | + | + | ± | + | + | − | ± | + | + | + | + | + | + | −* | −* | × |
| | B. pyocyaneus | + | + | + | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | B. dysenteriæ (Shiga) | −* | × | + | −* | ± | + | × | ± | + | − | −* | + | −* | ± | + | + | + | + | + | + | + | ± | + | + | + | + | + | + | + | + | ± | + | + |
| | B. dysenteriæ (Flexner) | × | ± | + | − | × | + | −* | ± | + | ± | ± | + | −* | ± | + | + | + | + | ± | + | + | + | + | + | + | + | + | + | + | + | × | + | + |
| | B. dysenteriæ (Mt. Desert) | ± | + | + | − | ± | + | + | + | + | × | ± | + | ± | + | + | + | + | + | ± | + | + | + | + | + | + | + | + | + | + | + | ± | + | + |
| | B. typhosus | + | + | + | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | . | . | . | + | + | + | + | + | + | + | + | + | ± | ± | + |
| | B. paratyphosus, A | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | B. paratyphosus, B | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | B. enteriditis (Gaertner) | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | B. coli communis | ± | ± | ± | ± | + | + | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | B. coli communior | ± | + | + | ± | + | + | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | Friedländer's bacillus | ± | + | + | × | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | B. lactis aerogenes | + | + | + | ± | ± | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ± | + | + |
| | B. ozænæ | × | ± | + | − | − | + | . | . | . | + | + | + | × | ± | + | + | + | + | −* | + | + | + | + | + | + | + | + | + | + | + | × | ± | + |
| | B. pullorum | + | + | + | × | ± | + | . | . | . | ± | + | + | . | . | . | + | + | + | + | + | + | . | . | . | + | + | + | + | + | + | + | + | + |

+ = growth like control; ± = restrained growth; × = markedly restrained growth; − = no growth; −* = some growth developed later.

irregular restraint at 1 to 100,000, more marked or complete in some instances at 1 to 50,000. There was, however, no evidence of a grouping according to the different types of bacilli.

On *cresylechtviolett* the strain of pneumococcus failed to grow whereas other allied cocci grew. Forty strains of this group were tested. A variable restraint or inhibition of individual cultures was noted, but this had no relation to the subgroups.

In the dysentery group there is a general tendency for the paradysentery types to show less restraint than the Shiga type. The result on fuchsin seemed to show a definite difference between the types. The inhibition caused by fuchsin was also of interest because of its presence in Endo agar. This is the most commonly used medium for the isolation of dysentery and must, therefore, have been found satisfactory for this group; at least we know of no unfavorable criticism of the growth on this medium. The question arises as to how far the influence of the dye itself might be lost after its reduction by sodium sulphite to the leuko-base.

Table II gives the results on fuchsin agar with the available strains of dysentery and paradysentery. The table gives the range of growth observed in several repetitions.

It is probable that some of the cultures under similar names, but obtained from different institutions,[1] are duplicates. As we were unable to determine this positively they are given separately in the table. They act as probable controls on the degree of variation of growth in the presence of the dye.

It is evident from table II that the reaction to fuchsin is not a completely specific difference between the dysentery and paradysentery groups. There is a shading of one group into the other. There is, however, a marked tendency to a general group reaction.

Two strains, one of the Shiga type and the other of the paradysentery type, which reacted almost alike were plated and ten fishings of each made in broth. These were planted on fuchsin agar to determine the probable range of variation under identical conditions. A moderate variation was noticed in some instances slightly greater than those already noted in the table.

[1] We are indebted to the Museum of Natural History, to The Rockefeller Institute for Medical Research, and to Professor Hiss and Dr. Torrey for these cultures.

TABLE II.

*Growth of Dysentery Bacilli on Fuchsin Agar.*

| Culture. | Mannite fermentation. | Time of incubation and dilution of dye. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 18 to 24 hours. | | | | 48 hours. | | | | 72 hours. | | |
| | | 25,000 | 50,000 | 100,000 | 150,000 | 25,000 | 50,000 | 100,000 | 150,000 | 25,000 | 50,000 | 100,000 |
| Shiga, Gay-Shiga, Shiga | − | − | − | − | − or × | − | − | s.c. or × | ± | − | s.c. | ± |
| Kruse, Kruse | − | − | − | − or s.c. | − or × | − | − | s.c. or × | ± | − | s.c. or × | ± |
| Kruse, Shiga | − | − | − | − or × | × | − | − | × or ± | ± | − | × | + |
| Shiga | − | − | − | − or × | ± | − | − or s.c. | × or ± | ± | − | ± | + |
| Torrey 21 | − | − | − | × or ± | ± | − | × or ± | + | + | − or 1 c. | × or ± | + |
| Cruz 1 | − | − | − or × | ± or + | + | − or × | ± | + | + | − or × | ± | + |
| Sydenham | + | − | − or × | ± or + | + | − | × or ± | + | + | − | ± | + |
| Torrey 4, Torrey 5–6, Hiss Y | + | − | × | ± or + | + | s.c. or × | ± or + | + | + | × or ± | ± | + |
| Hiss Y, Flexner-Harris, Medburg, Ferra-Hiss, Torrey 16–3, Torrey 32, V, Baltimore, Strong, Flexner, Mt. Desert | + | × | ± or + | ± or + | + | × or ± | ± or + | + | + | ± | ± or + | + |
| Strong, Gay-Flexner, Silk, Baltimore, Flexner, Gray, Torrey 3, Torrey 41, Flexner-Harris | + | ± | ± or + | + | + | ± or + | + | + | + | ± or + | + | + |
| Gray B, Hiss 48 | + | + | + | + | + | + | + | + | + | + | + | + |

− = no growth; 1 c. = colony; s.c. = several colonies; × = very slight growth; ± = evident restraint; + = practically like control.

*Influence of Decolorization on Fuchsin.*—An agar was made containing decolorized fuchsin diluted 1 to 25,000. The strains showing no growth in this concentration of the unaltered dye after twenty-four hours' incubation were planted. Three strains grew as well as on the control, four strains showed slight to moderate restraint, and one strain showed marked restraint. With a similar medium containing the decolorized dye in a dilution of 1 to 50,000, one strain showed some restraint, but at 1 to 100,000 no restraint was evident with any of the strains.

Evidently decolorization robs the dye of some of its action. A series of plates was made containing the decolorized dye in the 1 to 50,000 dilution. These were kept at room temperature and in the light to bring back the color gradually. Several of these plates, with controls, were planted each day. When planted the day after making, some restraint was evident. Those planted thereafter gave no growth except with a strain which grew in the presence of undecolorized fuchsin. The reaction of three members of the dysentery group to the violet dyes has been given. Because of the results on fuchsin the reaction of all the strains was tried, gentian violet and dahlia being used. With both dyes, individual strains showed more variation than with the fuchsin. There was no apparent difference between the dysentery and paradysentery group, the individual strains of both groups showing, however, markedly different degrees of restraint in the presence of the dye.

*Reaction of the Streptococcus-Pneumococcus Group to Dyes.*—In the results already given there is a marked quantitative difference in the reaction of the streptococcus-pneumococcus group as compared with the other Gram-positive bacteria. Because of this about a hundred strains of the group were tried;[2] these included typical pneumococci, streptococci, *Streptococcus mucosus,* types from chronic endocarditis, streptococci from various diseased conditions in man, a series isolated from the conjunctiva, a series isolated from domestic animals, and a few from the external world. Two dyes were used, gentian violet and Hoffman violet. The results were practically the same with both dyes.

[2] We are indebted to Miss Jean Broadhurst for most of the streptococcus cultures.

In dilutions of 1 to 500,000 of the dye the members of the group grew as freely as on the control, with a few exceptions where slight restraint or delayed growth was evident. By increasing the concentration of the dye, the amount of restraint becomes greater, and some of the strains fail to grow, the number increasing with the concentration. When a dilution of 1 to 100,000 is reached a few strains still grow but the growth is restrained or delayed. One exception was a strain of *Streptococcus lacticus,* which even at 1 to 600,000 showed inhibited or delayed and feeble growth.

The Gram-positive organisms used for comparison, including sixteen strains of staphylococci, failed to grow at 1 to 500,000 dilution of the dye. In a few instances a slight growth developed along the edge of the slant, probably due to the dilution of the dye by the water of condensation.

The streptococcus-pneumococcus group, even the feebly growing strains, is, therefore, more resistant to the action of the violet dyes than other Gram-positive bacteria, growing at dilutions which still inhibit the other Gram-positive bacteria. This may be found of value in isolation from mixed material.

The above observations explain the irregular results in the streptococcus-pneumococcus group given in Churchman's protocols. Stowell, Hilliard, and Schlesinger (4) found regular inhibition of nearly all their strains.

The influence of decolorization by sodium sulphite on the restraining action of gentian violet was very slight. In a concentration of 1 to 50,000 or 1 to 100,000 of the dye, there was no growth of either the staphylococcus or hay bacillus; when a dilution of 1 to 400,000 was reached, there was very feeble growth of the staphylococcus, but not of the hay bacillus.

While the above work was in progress, a further article by Churchman appeared (5). He found similar results, using allied dyes. As he used only four strains for testing, and, as far as we can determine, only one dilution of the dye, he naturally found none of the variations in individual dyes, in which we were especially interested.

SUMMARY.

Gentian violet and allied anilin dyes have a similar influence on bacterial growth, dividing bacteria into two groups corresponding in general to their reaction to the Gram stain.

Among Gram-negative bacteria a strain is occasionally encountered which will not grow on violet agar, differentiating it from other members of the same species or variety.

The reaction is quantitative, although the quantitative character is more marked with some species than with others.

The streptococcus-pneumococcus group differ from other Gram-positive bacteria in their ability to grow in the presence of amounts of dye sufficient to inhibit the other species.

The dysentery bacillus group shows marked variation in the presence of dyes. In the case of fuchsin the variation approaches closely a specific difference between the dysentery and paradysentery groups. The variations of the latter groups with other dyes show no correlation with the common differential characteristics. A closer study might reveal variations in other characteristics which would parallel the different reactions to dyes. Decolorization with sodium sulphite robs the dyes of some of their inhibitive powers.

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# THE ACID AGGLUTINATION OF PNEUMOCOCCI.*

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The method of agglutination of bacteria by acid was introduced by Michaelis (1) for the differentiation of bacterial species. By means of it closely allied types can be distinguished, since the hydrogen ion concentration at which agglutination is maximal is characteristic for the various species.

The determination of the point of maximal agglutination has been compared to the determination of the melting point of a chemical compound. However, it is not possible to determine accurately the point of maximum agglutination for all cultures. On the one hand, no agglutination at any hydrogen ion concentration may occur. This has been found by Beniasch (2) to be the case for *Bacillus coli,* and, indeed, certain strains of nearly all species of bacteria have been found by Beniasch to be non-agglutinable within the tested reaction limits. On the other hand, agglutination may occur in uniform degree over a somewhat broad range of hydrogen ion concentration.

The result of much work on the acid agglutination of the typhoid-colon group of bacilli has shown that *Bacillus typhosus* and *Bacillus paratyphosus* are easily distinguished by means of the reaction. Differences between the paratyphoid bacilli A and B, or C, have not been seen by most workers (Beniasch (2), Jaffé (3), and Heimann (4)).

The writer wished to learn whether certain types of pneumococci (described by Dochez and Gillespie (5)), which are distinguished sharply by means of serum reactions, but incapable of being distinguished morphologically or by cultural tests, can be distinguished and classified by means of acid agglutination.

## THE METHOD OF ACID AGGLUTINATION.

To different samples of bacterial emulsion in distilled water, different hydrogen ion concentrations are imparted, usually varying in

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geometrical progression. The emulsions are incubated at body temperature. Agglutination should occur in some mixtures, and if an optimum is to be recorded, some mixtures should prove to be too acid and others not sufficiently acid for the occurrence of the phenomenon.

The reactions of the bacterial suspensions are controlled by adding to them solutions of a weak acid, or of an acid still further weakened by the presence of one of its salts. By this means low hydrogen ion concentrations are given by relatively high concentrations of acid, so that loss of small quantities of acid in neutralizing alkaline substances from the culture medium is unimportant. In either case the acid concentration is varied in the series of bacterial emulsions; if salt is added the same concentration must obtain in all members of the series. The two equations used for calculating the hydrogen ion concentrations differ only because they are approximations, as the derivation shows.

Applying the mass action law to the electrolytic dissociation of the monobasic acid whose constant is k, we have,

$(H')\cdot(R^-) = k(HR)$ where (HR) indicates the concentration of the acid, in gram molecules per liter, (H′) the concentration of hydrogen ions, and ($R^-$) the concentration of the R ions. (R) includes all R ions from whatever source. If we increase the concentration of R ions in a solution of the pure acid HR by adding a salt NaR, then we have a molecule of $R^-$ for every molecule of salt introduced, since the salt is almost completely dissociated; and though the dissociation of the acid retrogresses we must have a molecule of $R^-$ for every molecule of H′ split off from the acid. We have, therefore,

$(H')\cdot(\text{salt} + H') = k\,(HR)$ (where salt = the number of molecules of salt introduced per liter)

$= k\,(\text{acid})$ (where acid = the number of molecules of acid introduced),

because the acid is mostly in the undissociated state. Therefore

$$(H') = k\ (\text{acid})/(\text{salt} + H').$$

Now if no salt is present this reduces at once to

$$(H') = \sqrt{k\,(\text{acid})}, \qquad (1)$$

whereas if salt is added even in moderate amounts, (H′) is then small in comparison with (salt), and the equation becomes

$$(H') = k\ (\text{acid})/(\text{salt}). \qquad (2)$$

Equation (2) shows that with this type of regulator dilution does not change, within limits, the hydrogen ion concentration. The k for acetic acid is $1.8 \cdot 10^{-5}$; for lactic acid it is $1.38 \cdot 10^{-4}$. The hydrogen ion concentration is given in grams per liter.

## TECHNIQUE.

All the organisms studied had recently been isolated from patients having lobar pneumonia.

The broth used for the tests was a beef infusion containing 0.5 per cent. of sodium chloride and 1 per cent. of Witte's pepton (Kahlbaum), sterilized intermittently in streaming steam for short periods. The reaction was + 0.6 per cent. acid to phenolphthalein.

The lactate mixtures were prepared from stock solutions of one third normal sodium lactate, with a small crystal of thymol, and normal lactic acid, according to the scheme which follows. They were employed fresh.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| N/3 sodium lactate in c.c. | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| N lactic acid in c.c. | $\frac{0.12^1}{8}$ | $\frac{0.25}{8}$ | $\frac{0.5}{8}$ | $\frac{1.0}{8}$ | $\frac{2.0}{8}$ | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | 16.0 |
| Distilled water in c.c. | 18.4 | 18.2 | 18.0 | 17.5 | 16.5 | 18.0 | 17.5 | 16.5 | 14.5 | 10.5 | 2.5 |
| Ratio of acid to salt | $\frac{1}{32}$ | $\frac{1}{16}$ | $\frac{1}{8}$ | $\frac{1}{4}$ | $\frac{1}{2}$ | 1 | 2 | 4 | 8 | 16 | 32 |
| H ion concentration in grams per liter, multiplied by $10^4$ | 0.04 | 0.1 | 0.2 | 0.4 | 0.7 | 1.4 | 2.8 | 5.5 | 11 | 22 | 44 |

A series of dilute lactates (1 to 6) was also used. It was prepared by adding five volumes of distilled water to one volume of each of the lactate mixtures described above.

Normal acetic acid, when diluted (1 to 1.3) by bacterial emulsion in the manner to be described, gives a reaction somewhat less acid than the 32:1 lactate mixture. It was used, and its reaction was recorded as if it were exactly equivalent to that lactate mixture. By diluting normal acetic acid 1 to 2.86 a reaction mixture can be obtained which when diluted by the bacterial emulsion corresponds exactly to the 16 to 1 lactate mixture. By making progressive dilutions from this N/2.86 acetic acid, diluting 1 to 4 in each case, a series of mixtures was obtained corresponding, when bacterial emulsion is added, to

[1] Read 0.12 c.c. of normal acid freshly diluted 1:8.

the lactate mixtures. In all, six different strengths of acetic acid were used.

In two experiments a series of mixtures of sodium acetate and acetic acid was used, each mixture being 1 to 257 normal with sodium acetate. Such solutions are not generally to be recommended. In one other case the salt content was different (table III, footnote 12).

Young cultures were produced by inoculating broth flasks taken cold from the refrigerator and by placing the flasks in the thermostat at 37.5° C. for eighteen hours or less. Cultures thus obtained showed well staining Gram-positive diplococci and short chains of good form. Except in experiment III such young cultures were always used for the tests. All cultures used were carefully examined and found pure. The cultures were whirled at high speed in a centrifuge and the supernatant fluid was poured off, leaving the deposit of cocci with only a small amount of adhering fluid. The cocci were then well emulsified in a volume of distilled water equal to about one tenth of that occupied by the original culture. 0.3 of a cubic centimeter of this emulsion was placed in each of a series of tubes in a rack, and one cubic centimeter of the proper reaction mixture of the regulator to be employed was added to each tube. Tubes containing acetic acid were stoppered. The rack of tubes was then shaken a few times in a standardized manner, because it was found that shaking markedly aids the agglutination, and was then placed in a water bath at about 37.5° C. until agglutination occurred.

## EXPERIMENTATION.

The writer found at once that the strains of pneumococcus at his disposal were not readily agglutinated by the acid-salt mixtures (acetic acid, also lactic acid) generally used for the test. Some strains showed no agglutination even after several days at body temperature. Michaelis states that the presence of salts inhibits the acid agglutination of bacteria, but that the effect of salts is slight in concentrations below 1/40 normal. Other investigators apparently have not studied this matter further. The following experiment was performed to see whether in the case of pneumococcus salts can be active in unusually low concentrations.

EXPERIMENT I.

*Inhibiting Action of Salts in Low Concentrations.*

*Pneumococcus 1.*

| Regulator. | Salt content. | Mixtures incubated. | Ratio acid : salt (lactate). 1/8 | 1/4 | 1/2 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactates | N/52 | 21 hrs. | − | − | − | − | − | − | − | − | − |
| Lactates diluted 1:6 | N/312 | 3 hrs. | − | − | − | − | + | +± | ± | − | − |
| Equivalent acetic acid | None added | 3 hrs. | − | − | − | − | − | ++ | ++ | − | − |

*Pneumococcus 29.*

| Regulator. | Salt content. | Mixtures incubated. | Ratio acid : salt (lactate). 1/8 | 1/4 | 1/2 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lactates | N/52 | 21 hrs. | − | − | − | − | − | − | − | − | − |
| Lactates diluted 1:6 | N/312 | 21 hrs. | − | − | − | − | − | − | − | − | − |
| Equivalent acetic acid | None added | 3 hrs. | − | − | − | − | − | − | − | + | − |
| | | 21[2] hrs. | | | | − | − | − | + | + | − |

This experiment shows plainly that the pneumococcus is sensitive to the action of salts in inhibiting acid agglutination even at a normality of 1/312 of salt. This was seen many times in later experimentation.

EXPERIMENT II.

*Effect of Washing Pneumococci in Distilled Water.*

*Pneumococcus 75.*

*All Mixtures Were Incubated for Twenty-Eight Hours.*

| | Ratio acid : salt (lactate). 1/8 | 1/4 | 1/2 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|---|---|
| Lactate mixture | | | | | | | | | |
| Unwashed | − | − | − | − | − | − | − | − | − |
| Washed | − | − | − | − | − | ± | − | − | − |
| Lactates diluted 1:2.5 | | | | | | | | | |
| Unwashed | − | − | − | − | − | ± | − | − | − |
| Washed | − | − | − | − | − | + | − | − | − |
| Lactates diluted 1:6 | | | | | | | | | |
| Unwashed | − | − | − | − | + | + | + | − | − |
| Washed | − | − | − | ± | ± | +± | +± | − | − |
| Equivalent acetate mixtures | | | | | | | | | |
| Unwashed | − | − | − | − | ± | + | + | ± | − |
| Washed | − | − | − | − | − | +± | ++ | ± | − |

The technique was now controlled to see whether washing the deposit of pneumococci would be a real refinement.

[2] Autolysis took place in the first three tubes during this time.

This experiment shows that no less acid is required to produce a given result[3] when the cocci are washed. Unwashed, though well drained, bacterial deposits were therefore used for all the tests that follow.

Certain results obtained by Dr. Hanes in this laboratory, when compared with the results of the writer, suggested that cultures incubated a few hours longer than was the practice in this investigation agglutinated well in the presence of the usual amount of salt, but at less acid reactions. A flask of broth was therefore inoculated with pneumococcus I and from it in twenty-four hours material was taken and inoculated into a second flask. Both flasks were then incubated for about eighteen hours. Smears showed that the old culture contained many Gram-negative as well as Gram-positive diplococci, whereas the young culture contained only Gram-positive organisms. The acid agglutination test, with undiluted lactates, gave the following results.

EXPERIMENT III.

*Age of the Culture.*

| Culture. | Mixtures incubated. | Ratio acid : salt. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 4 | 8 | 16 | 32 |
| Young... | 6 hrs. | − | − | − | − | − | − |
| Young... | 20 hrs. | − | − | − | − | − | − |
| Old ..... | 1 hr. | − | − | − | − | − | − |
| Old ..... | 2 hrs. | − | − | ± | − | − | − |
| Old ..... | 6 hrs. | − | + | + | − | − | − |
| Old ..... | 20 hrs. | − | +± | ++ | − | − | − |

It will be seen that the old culture agglutinates well in six hours, though the young culture shows nothing in twenty hours, but that the optimum in the case of the old culture is different from that characteristic of the strain and of other organisms of the type (experiment I and table I). The optimum seen here for the old culture coincides with that which Beniasch found for the pneumococcus.

With the technique given above, various strains of pneumococci were now examined by the method of acid agglutination. In accordance with a classification already made for these strains by the

[3] The power of acid regulators to maintain the calculated hydrogen ion concentration in the presence of traces of medium decreases with decreasing concentration of acid in the regulating mixture.

use of immune sera, by protection of mice by the simultaneous administration of serum with living cocci and by agglutination (Dochez and Gillespie (5)), the material is divided into three groups and presented in tables I, II, and III. Many of the strains were at once tested with three regulators (lactates, lactates 1 to 6, and acetic acid). All strains were tested at least once with the undiluted lactates, and when the result was negative a lower salt concentration was tried by the use of the diluted lactates or acetic acid. No strain failed to agglutinate with the acetic acid mixture. Negative results, which were always attributable to too high a salt content of the regulator, have not been recorded in the tables.

TABLE I.

*Acid Agglutination of Pneumococci of Type 1 (Neufeld 1).*

| Culture.[4] | Regulator.[5] | Hours.[6] | Hydrogen ion concentration.[7] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.2 | 0.4 | 0.7 | 1.4 | 2.8 | 5.5 | 11.0 | 22.0 | 44.0 |
| 1.58[13] | Lactate 1:6 | 45 | − | − | − | − | − | ± | ± | − | − |
| 1.58[16] | Lactate 1:6 | 2 | | | | − | − | + | − | − | − |
| 1.58[16] | Acetic acid | 2 | | | | − | − | + | + | − | − |
| 1.64[7] | Lactate 1:6 | 3 | − | − | − | − | + | +± | ± | − | − |
| 1.64[7] | Acetic acid | 3 | − | − | − | − | − | ++ | ++ | − | − |
| 1.67[7] | Acetic acid | 1 1/2 | | | | − | − | + | + | − | − |
| 88.0[16] | Lactate | 6 1/2 | − | − | − | − | − | ± | ± | − | |
| 75.6[3] | Lactate 1:6 | 28 | − | − | − | − | ± | + | ± | − | − |
| 75.6[3] | Acetate | 28 | − | − | − | − | ± | + | + | ± | − |
| 94.1[7] | Lactate | 50 | − | − | − | − | + | + | − | − | − |
| 94.1[7] | Lactate 1:6 | 4 | − | − | − | − | − | +± | ± | − | − |
| 43.5[19] | Lactate 1:6 | 5 1/2 | | | | − | − | + | +± | − | − |
| 43.5[19] | Acetic acid | 1 3/10 | | | | − | − | + | +± | − | − |
| 73.1[10] | Lactate 1:6 | 20 | | | | − | − | ± | + | − | − |
| 73.1[10] | Acetic acid | 20 | | | | − | − | ± | ++ | − | − |
| 73.1[12] | Acetic acid | 1 3/10 | | | | − | − | + | +± | − | − |
| 90.1[10] | Lactate 1:6 | 1 3/10 | | | | − | − | ± | ± | − | − |
| 90.1[10] | Acetic acid | 1 3/10 | | | | − | − | + | +± | − | − |
| 90.1[11] | Lactate 1:6 | 1 1/2 | | | | ± | + | + | + | − | − |
| 90.1[11] | Acetic acid | 1 1/2 | | | | − | ± | + | + | − | − |
| 44.6[15] | Lactate 1:6 | 1 1/5 | | | | − | − | +± | + | − | − |
| 44.6[15] | Acetic acid | 1 1/5 | | | | − | − | ++ | ++ | − | − |

[4] The integral part of the laboratory number specifies the strain, the decimal gives the number of animal passages, and the exponent gives the number of transfers since the last animal passage.

[5] These regulators have been described above. Lactate means the sodium lactate-lactic acid mixtures; lactate 1:6 means such mixtures freshly diluted six times with distilled water; acetate means the sodium acetate-acetic acid mixtures.

[6] Time required for the recorded degree of agglutination.

[7] Multiplied by $10^4$. The unit is a gram per liter.

In table I are given the results for eight strains of pneumococci of the No. 1 (or Neufeld) type. Only two positive results, and these were weak, were obtained with the undiluted lactate mixtures. This type shows narrow zones of agglutination with an optimum at a hydrogen ion concentration of 5.5 to 11 times $10^{-4}$ grams per liter. This optimum is therefore a little different from that found by Beniasch (2) for pneumococci.

TABLE II.

*Acid Agglutination of Pneumococci of Type 2.*

| Culture. | Regulator. | Hours. | Hydrogen ion concentration.[8] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.2 | 0.4 | 0.7 | 1.4 | 2.8 | 5.5 | 11.0 | 22.0 | 44.0 |
| 2.10[5] | Acetic acid | 2 3/5 | | | | − | − | − | − | + | ± |
| 2.10[17] | Acetic acid | 1 1/2 | | | | − | − | − | + | + | − |
| 95.0[5] | Lactate | 17 | − | − | − | − | − | − | ± | ± | ± |
| 95.0[7] | Lactate 1:6 | 50 | − | − | − | − | − | ± | + | + | + |
| 85.6[9] | Lactate 1:6 | 4 | − | − | − | − | − | − | + | − | − |
| 85.6[11] | Lactate 1:6 | 20 | | | | − | − | ± | ± | + | ± |
| 85.6[11] | Acetic acid | 2 | | | | − | − | − | + | + | − |
| 85.6[13] | Acetic acid | 3 | | | | − | − | − | + | − | − |
| (Same, read later) | | 22 | | | | − | − | − | ++ | + | ± |
| 15.7[12] | Acetic acid | 1 | | | | − | − | − | + | + | − |
| 23.6[11] | Acetic acid | 1 | | | | − | − | − | ± | ± | − |
| 39.5[8] | Acetic acid | 5 1/2 | | | | − | − | − | +± | + | − |
| 29.7[12] | Acetic acid | 3 | | | | − | − | − | − | + | − |
| 89.1[9 9] | Lactate | 1 | | | | + | + | + | + | ± | − |
| 89.1[9] | Lactate 1:6 | 1 | | | | + | + | + | + | ± | − |
| 89.1[9] | Acetic acid | 1 | | | | ± | +± | +± | + | ± | − |
| 89.2[8] | Lactate | 1/5 | | | − | ± | ± | + | + | ± | |
| 89.2[9] | Lactate | 1/5 | − | ± | ± | ± | ± | + | + | + | |

Table II gives the results for eight strains of pneumococci of a different serological type (No. 2). With the exception of No. 89 these strains showed weak or no agglutination with the undiluted lactate mixture. Some even gave negative results with the diluted lactates. With the same exception the strains show a narrow zone of agglutination with an optimum of 11 to 22 times $10^{-4}$ grams of hydrion per liter. This lies within the region found by Beniasch to be characteristic for streptococci.

[8] Multiplied by $10^{4}$.

[9] Pneumococcus 89 has constantly shown atypical forms in cultures. Its behavior with serum 2 is, however, perfectly typical.

TABLE III.

*Acid Agglutination of Atypical Strains of Pneumococci.*

| Culture. | Regulator. | Hours. | Hydrogen ion concentration.[10] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.04 | 0.1 | 0.2 | 0.4 | 0.7 | 1.4 | 2.8 | 5.5 | 11.0 | 22.0 | 44.0 |
| 71.1[10] | Lactate | 17 | | | | | | − | − | − | + | + | + |
| 71.1[10] | Lactate 1:6 | 1 | | | | | | − | − | − | + | + | + |
| 71.1[10] | Acetic acid | 1 | | | | | | − | − | − | + | + | + |
| 38.6[8] | Lactate | 1 1/5 | | | | | | − | − | + | + | + | + |
| 38.6[8] | Lactate 1:6 | 1 1/5 | | | | | | − | − | + | + | + | + |
| 36.16[3] | Lactate | 1 | | | | | | − | − | + | + | + | + |
| 36.16[3] | Lactate 1:6 | 1 | | | | | | − | ± | + | + | + | + |
| 36.16[3] | Acetic acid | 1 | | | | | | − | − | + | + | + | + |
| 55.6[11 11] | Lactate | 1 1/5 | | | | | | − | ± | + | + | + | ± |
| 55.6[11] | Lactate 1:6 | 1 1/5 | | | | | | ± | + | + | + | + | + |
| PaS.1[2] | Lactate | 1 1/2 | | | | | | + | + | + ± | + ± | + ± | + |
| PaS.1[2] | Acetic acid | 1 1/2 | | | | | | — | + | + ± | + + | + | + |
| 62.0[8] | Lactate | 2 | | | | | | + | + | + | + | + | + |
| 62.0[8] | Acetic acid | 2 | | | | | | ± | + | + | + | ± | − |
| 37–8.6[5] | Lactate | 1 1/5 | | | | | | + | + ± | + ± | + | ± | − |
| 37–8.6[5] | Acetic acid | 1 1/5 | | | | | | — | + ± | + ± | + | ± | − |
| 34.6[15] | Lactate | 22 | | | | | | + | ± | − | − | − | − |
| 34.6[17] | Lactate | 45 | | | − | ± | + | + | − | − | | | |
| 76.4[11] | Lactate | 2 | | | | | | + | − | − | − | − | − |
| 76.4[11] | Acetic acid | 2 | | | | | | + | ± | − | − | − | − |
| 76.4[11] | Lactate | 1 3/10 | | | + | + + | + ± | ± | − | | | | |
| 82.5[9] | Lactate | 22 | | | | | | ± | − | − | − | − | − |
| 82.5[11] | Lactate | 1 1/2 | | | + + | + + | ± | — | − | − | | | |
| 52.2[9] | Lactate | 6 1/2 | | | | | | + | ± | − | − | − | − |
| 52.2[13] | Lactate | 1 3/10 | + | + | + ± | + | ± | ± | − | − | | | |
| *Streptococcus mucosus.* | | | | | | | | | | | | | |
| 49.3[9] | Lactate | 2 | | | + ± | ± | − | − | − | − | − | − | |
| 19.9[5] | Acetate[12] | 1 8/10 | | | − | + + | + + + | + + + | + + | − | | | |

Table III contains the results for ten strains belonging to neither type 1 nor 2, and not showing any considerable degree of relationship one to another by serum tests; for one strain (No. 55) which showed relationship with type 2 by the protection test, but not by the agglutination test; and for two strains of *Streptococcus mucosus,* one of which (No. 49) has all the properties of a streptococcus except that it has a large capsule and produces a mucous exudate in mice, the other of which (No. 19) has all the typical properties of a pneumococcus except for its extra large capsule, mucoid growth on

[10] Multiplied by $10^4$.

[11] Pneumococcus 55 belongs with type 2 by protection test, but it failed to agglutinate with serum 2.

[12] The concentration of sodium acetate was in this case N/26.

agar, and production of a mucous exudate in mice. The agglutination of most of these organisms is rapid and is never inhibited by salts in the same manner as is that of pneumococci of types 1 and 2. The zones of agglutination are for the most part broad; none are coincident with those characteristic of types 1 and 2.

## SUMMARY.

Eight strains of pneumococci of serological type 1, eight strains of type 2, and eleven strains belonging to neither type have been tested by the method of acid agglutination.

Strains belonging to the two typical groups have, as a rule, narrow zones of agglutination. The optimum hydrogen ion concentrations are different in the two cases. Other pneumococci have broad zones or, in a few cases, narrow zones not coincident with those occupied by the typical organisms.

The agglutination of most of the pneumococci of types 1 and 2 is extremely susceptible to the inhibiting action of salts. This is not true of the other pneumococci.

Old broth cultures may show an optimum hydrogen ion concentration different from that shown by young broth cultures.

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# AN IMMUNOLOGICAL STUDY OF PNEUMOCOCCUS MUCOSUS.*

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Although the older literature, reviewed by von Lingelsheim (1), contains a number of descriptions of encapsulated streptococci, the importance of organisms of this type as frequent producers of disease has only been fully recognized since the publications of Schottmüller (2), who found a well characterized organism, which he called *Streptococcus mucosus,* in various pyogenic processes and in lobar pneumonia. Many observers have since encountered this organism, occurring as it does in the most diverse pyogenic lesions, and its morphologic and biologic characteristics are well known.

Among seventy-four cultures isolated from patients with lobar pneumonia in the Hospital of The Rockefeller Institute in the course of the past two years, nine, or 12 per cent., have been classed provisionally as belonging to the group of encapsulated streptococci of Schottmüller. These organisms, through their power of producing a tenacious mucoid exudate in the peritoneal cavity of white mice, were readily separated from the larger group of encapsulated diplococci. At the same time, however, their cultural characteristics made it seem probable that they represented a variety of the pneumococcus rather than that they belonged to the streptococcus group.

With the hope of determining more exactly the relations of *Streptococcus mucosus* of Schottmüller to *Diplococcus pneumoniæ,* on the one hand, and to the streptococci, on the other, six of our nine organisms were selected for a comparative study of this problem. These six organisms were chosen at random from the nine in our possession. For convenience I shall refer to this group of organisms as *Pneumococcus* rather than *Streptococcus mucosus.* They were reobtained for this study from the dried spleens[1] of white mice which had died following a peritoneal injection of the sputum from

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[1] It not infrequently happens that mice infected intraperitoneally with pneumococci show, in addition to a pneumococcus septicemia, an infection with the

patients suffering from lobar pneumonia, the spleens having been preserved *in vacuo* for several months (Neufeld and Händel (3)). Two other pneumococci and two organisms of the streptococcus group were selected for study in addition to these six. The distinguishing characteristics of all the organisms are given for comparison in table I.

The six representatives of the *Pneumococcus mucosus* group (Nos. 19, 26, 42, 54, 68, and 96) possess identical properties, and these will be described in detail. Of the two other pneumococci, one is an organism secured from Dr. Neufeld and is the type representative of Dochez's (4) group I; the other belongs to Dochez's group II. One of the streptococci (No. 174) is a typical hemolyzing *Streptococcus pyogenes,* while the other (No. 49) is exactly described by the name *Streptococcus mucosus.* This organism was selected for comparative study because it possesses a distinct capsule and has the power of producing a mucoid exudate in the peritoneal cavity of a white mouse, having at the same time the cultural and morphological characters of a streptococcus. Its characteristics, as detailed in table I, would seem to place it nearer the streptococci than the pneumococci. It was recovered from the sputum of a nonfatal case of lobar pneumonia.

As stated above, the six members of the group of *Pneumococcus mucosus* chosen for this study exhibit identical properties; they likewise conform to Schottmüller's description of *Streptococcus mucosus.* There can be no doubt, then, that they belong to the same group as the organisms he described, and a brief description of this group will be given.

*Pneumococcus mucosus* occurs in diplococcal form, often arranged in short chains of from four to about sixteen members. These are surrounded by a broad mucoid capsule which, in purulent exudates,

bacillus of mouse typhoid (*B. typhi murium*). Both organisms are often present in the blood, but this may show only pneumococci, while smears of the exudate from the peritoneal cavity show that both are present. Under such conditions the dried spleens in which the pneumococci are preserved are contaminated with *B. typhi murium,* and the recovery of the pneumococcus in pure culture is very difficult. Several of our spleens were thus contaminated, and other methods of purification having failed, we resorted with complete success to the production in rabbits of an anti-mouse typhoid serum. These sera will prevent the growth of the mouse typhoid in the animal body, whereas pneumococci develop as usual.

TABLE I.

| Organism. | Morphology. | Solubility in sodium taurocholate. | Growth on blood agar plates. | Plain agar. | Litmus-milk. | Bouillon. | Hiss's serum inulin medium. | Exudate in peritoneal cavity of mice. |
|---|---|---|---|---|---|---|---|---|
| Six strains of *Pneumococcus mucosus* (Nos. 19, 26, 42, 54, 68, 96) | Diplococci single and in short chains; cocci round or slightly flattened, and possessing broad, mucoid capsules | Soluble | Large round drop-like mucoid colonies, colorless and translucent; greenish brown discoloration of medium | Very delicate, scanty, translucent growth; media not clouded | Acid and coagulated in 24 hrs. | Moderately turbid; very slight sediment | Acid and coagulated in 24 hrs. | Mucoid, tenacious, easily drawn out into thread |
| Pneumococcus I and II | Lancet-shaped diplococci with delicate capsules | Soluble | Delicate, discrete, drop-like colonies; greenish brown discoloration of medium | Very delicate, scanty, translucent growth; media not clouded | Acid and coagulated in 24 hrs. | Moderately turbid; very slight sediment | Acid and coagulated in 24 hrs. | Not mucoid or tenacious. |
| Organism 49, *Streptococcus mucosus* | Small round cocci arranged in short or long chains | Not soluble | Grayish translucent colonies | Abundant growth; media not clouded; surface rather dry | Acid and coagulated in 24 hrs. | Turbid; moderately heavy sediment | No acid production or coagulation | Mucoid and tenacious. |
| *Streptococcus longus* | Small round cocci arranged in chains | Not soluble | Grayish translucent colonies; wide area of hemolysis | Abundant small grayish colonies; media clouded | Acid and coagulated in 24 hrs. | Turbid; heavy sediment | No acid production or coagulation | Not mucoid or tenacious. |

merges almost imperceptibly into the characteristic tenacious material produced so abundantly by the growth of this organism in the animal body. The capsule persists in cultures upon artificial media and is always easily demonstrated. The organism is round or slightly flattened in shape and varies in size, even in the same culture. It gives the impression, especially when stained by Gram's method, of being larger and coarser than other pneumococci.

*Pneumococcus mucosus,* in common with other pneumococci, grows best upon a medium containing blood serum or whole blood. Growth is very scanty or absent upon plain agar, while relatively few of the transplanted organisms grow even upon a favorable medium such as blood agar. It is necessary, in order to secure luxuriant growth, to inoculate heavily any medium used. The cultures dry out rapidly, even under the favorable conditions of ice box temperature, and after ten days they are usually sterile. The individual colonies are round, moist, colorless, and translucent. When several colonies have become confluent, as they do very readily, they tend to run down the surface of the agar slant, presenting the appearance of a dependent drop of clear, tenacious, mucoid material. Smear cultures on blood agar present, as a rule, a smooth, homogeneous, translucent growth, which is colorless itself, but causes a greenish brown discoloration of the medium. With the platinum loop, the growth can be drawn out into short tenacious threads. Bouillon, if carefully prepared and heavily inoculated, produces a moderately luxuriant growth, with little or no tendency to sedimentation. Growth on gelatin is slight and there is no liquefaction. Litmus-milk turns red and coagulates solidly in twenty-four hours. Hiss's serum inulin medium is reddened and coagulated.

The organism dissolves readily in the presence of a solution of sodium taurocholate. If several drops of a 2 per cent. solution of sodium taurocholate are added to two cubic centimeters of a twenty-four hour growth in bouillon, the mixture rapidly becomes clear, provided there is no trace of blood serum present.

*Pneumococcus mucosus* is highly pathogenic for white mice and rabbits. One millionth of a cubic centimeter of an eighteen hour bouillon culture will kill a mouse in from fifteen to twenty-four hours when injected intraperitoneally. Its pathogenicity does not seem to be lessened by prolonged cultivation upon artificial media.

SPECIFIC AGGLUTINATION OF PNEUMOCOCCUS MUCOSUS.

It has been found by Dochez and Gillespie (4) that, by means of immunological reactions (agglutination and protection), the various strains of pneumococci recovered from the sputum and blood of patients with lobar pneumonia can be classified into at least three groups, with a fourth, less well defined group comprising organisms showing no interrelations. Their results demonstrate beyond question the heterogeneous character of the group of organisms possessing the physical and cultural properties of the pneumococcus. The macroscopic agglutination method was employed by Dochez, equal quantities of immune serum and twenty-four hour bouillon culture being used in the reaction. The results obtained were clean cut and definite, and were fully confirmed in each instance by the results of protection experiments, so that notwithstanding the low serum dilution employed, the method yielded specific information.

This method was therefore employed in attempting to demonstrate specific agglutinins for *Pneumococcus mucosus* in the serum of rabbits highly immunized to this organism. My results were uniformly negative. Two possibilities presented themselves. Either *Pneumococcus mucosus* was incapable of stimulating specific agglutinins, or else the method employed for their detection was inadequate. The first possibility seemed unlikely, since rabbits could be brought to a high state of active immunity to *Pneumococcus mucosus,*—higher indeed than rabbits immunized to other types of pneumococci and yielding good, specifically agglutinating sera.

A distinct analogy seemed to exist between the problem here presented and that of the specific agglutination of encapsulated bacilli; for the heavy mucoid capsule of *Pneumococcus mucosus* is its most strikingly distinguishing feature when compared with other pneumococci. The work of Porges (5) and von Eisler and Porges (6) has demonstrated that, contrary to the experience of previous workers, the capsule bacilli can be agglutinated readily by homologous immune sera, provided the bacilli are subjected previously to a preparatory treatment directed toward the destruction of their capsules.

The procedure recommended for this purpose by Porges has been employed with excellent results in the present attempt to demonstrate specific agglutinins in animals immunized to *Pneumococcus mucosus*. The method of Porges, as adapted to the present study, is as follows: Half a liter of a twenty-four hour bouillon culture of *Pneumococcus mucosus* was centrifugalized at high speed for half an hour, the supernatant fluid decanted off, and the organisms taken up in ten cubic centimeters of salt solution. Three cubic centimeters of this emulsion were added to each of three test-tubes and one cubic centimeter (one fourth volume) of N/4 hydrochloric acid was added to each. The tubes were placed in a water bath at 80° C., and one was removed in twenty minutes, the second in thirty, and the third in fifty minutes. They were placed immediately in ice water, and when thoroughly cooled one cubic centimeter of N/4 sodium hydrate was added to each tube. The emulsions thus prepared are neutral, they show no tendency toward spontaneous agglutination, and sedimentation takes place very slowly.

The six *Pneumococcus mucosus* organisms were treated after this fashion and the agglutinability of each was tested with the sera of six rabbits, each of which had been immunized to one of these organisms. The results are shown in table II.

TABLE II.

*Agglutination (Porges Method).*

| Organism No. | Immune sera. | | | | | | | | | | Normal rabbit serum. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19 | 26 | 42 | 54 | 68 | 96 | I | II | 49 | 174 | |
| 19 | + | + | + | + | + | + | − | − | − | − | − |
| 26 | + | + | + | + | + | + | − | − | − | − | − |
| 42 | + | + | + | + | + | + | − | − | − | − | − |
| 54 | + | + | + | + | + | + | − | − | − | − | − |
| 68 | + | + | + | + | + | + | − | − | − | − | − |
| 96 | + | + | + | + | + | + | − | − | − | − | − |
| I | − | − | − | − | − | − | + | − | − | − | − |
| II | − | − | − | − | − | − | − | + | − | − | − |

In every instance the treated organisms agglutinated, whereas twenty-four hour bouillon cultures of the same organisms constantly failed to agglutinate. Equal quantities of immune serum and emulsion were used, and the tubes were kept at 37° C. during the experiment. Agglutination appeared promptly as a rule, and readings

made at the end of one hour were usually positive; final readings were made after six hours. The end result is quite definite, the supernatant fluid being clear, with the organisms forming a loosely coherent mass at the bottom of the tube. Of the three tubes of emulsion which were heated for varying lengths of time, the one treated for fifty minutes agglutinated slightly better than the others. As controls, emulsions of two other pneumococci (Nos. I and II) were made as described above, and these were likewise treated with the six *Pneumococcus mucosus* sera and with their own specific immune sera. They failed to agglutinate with the former, although with its own specific serum each agglutinated promptly. The emulsions of *Pneumococcus mucosus* were in no case agglutinated by sera other than their own, and remained well suspended in normal sera.

These reactions leave no room for doubt that the method of Porges is efficacious in rendering *Pneumococcus mucosus* specifically agglutinable. This result is probably though not certainly attributable to the destruction of the heavy, mucoid capsules of the organisms. It is conceivable that other factors play a part but, at any rate, it can easily be demonstrated that the capsules have almost if not quite disappeared during the treatment.

### COMPLEMENT FIXATION EXPERIMENTS.

It is desirable to learn not only the relations of *Pneumococcus mucosus* to other pneumococci, but to the streptococcus group as well. Agglutination experiments demonstrated clearly the presence of specific agglutinins in the sera of rabbits immunized with *Pneumococcus mucosus,* but since the method employed is inapplicable to the streptococci, owing to their marked tendency toward spontaneous clumping and sedimentation, it throws no light upon a possible relationship of *Pneumococcus mucosus* to the streptococcus group.

The complement fixation method of Bordet-Gengou seemed much better suited to this purpose and was therefore used with the results detailed below. The slightly modified Wassermann technique, as described by Walker and Swift (7), was employed.

*Antigens.*—Antigens with strong fixing power and weak anticomplementary action are readily obtained with *Pneumococcus*

*mucosus* (8). Twenty-four hour bouillon cultures are thoroughly centrifuged, the organisms washed once in normal salt solution, this removed as completely as possible, and the residue dried *in vacuo* over sulphuric acid. The dried substance is now weighed, one cubic centimeter of salt solution containing 0.5 per cent. phenol is added for each milligram of the dried material, and the whole is shaken for twenty-four hours in a bottle containing glass beads. The resulting emulsion is centrifugalized at high speed and the clear or slightly opalescent supernatant fluid is pipetted into rubber-corked brown bottles and kept in the ice box. Under these conditions the antigens have shown no deterioration after more than three months.

Antigens were prepared from each of the six strains of *Pneumococcus mucosus* and from pneumococcus I and II. In addition, antigens were made in a similar fashion from the *Streptococcus mucosus* and *Streptococcus pyogenes* previously described.

*Immune Sera.*—Rabbits were immunized to all the various organisms mentioned above. *Pneumococcus mucosus* is highly pathogenic for rabbits and, to avoid losses, the greatest care is necessary in rendering them immune to it. It frequently happens that rabbits succumb to a dose only slightly larger than the previous one, even after weeks of treatment with carefully graded doses of the organism. With care, however, quite high degrees of active immunity can be obtained, so that animals will show no serious reaction after an intravenous injection of the bacteria from 100 cubic centimeters of twenty-four hour bouillon culture. When we recall that 0.000001 of a cubic centimeter will kill, this degree of immunity is seen to be considerable. Organisms should always be centrifugalized free from the bouillon medium, for it is harmful when injected repeatedly. It has been found best to give an initial injection of killed organisms from 30 to 50 cubic centimeters of bouillon, and then to use live organisms for the next injection, beginning with very small doses and increasing as rapidly as the animal's condition permits. It is desirable that the animals should react severely to the injections; those animals in my series which have shown high fever and considerable loss of weight have furnished the most powerful sera.

Guinea pig complement, sheep cells, and anti-sheep cell rabbit serum were employed as a hemolytic system.

The results of the complement fixation experiments are illustrated by the tables III to X, which have been selected as typical. The various immune sera were first tested against constant quantities (0.1 of a cubic centimeter) of the different antigens, the sera being progressively diluted, and then against diminishing quantities of antigens, 0.1 of a cubic centimeter of serum being used.

The amount of antigen used was never greater than one fifth of the anticomplementary dose. The uniformity in the strength of antigens prepared from the six strains of *Pneumococcus mucosus* has been striking. As an antigen, any one of them could be chosen as representative of the group.

Specifically fixing sera have been obtained, as a rule, without great difficulty. It has been easier to obtain sera with high fixing powers by immunizing with *Pneumococcus mucosus* than with the other types of organisms used. This may be due in part to the severe reactions which the *Pneumococcus mucosus* animals showed to the earlier injections.

TABLE III.

*Serum 26 in Diminishing Quantities with Constant Amount (0.1 Cubic Centimeter) of Antigen.*

| Antigen No. | 0.005 c.c. | 0.0025 c.c. | 0.0012 c.c. | 0.0006 c.c. | 0.0003 c.c. |
|---|---|---|---|---|---|
| 26 (0.1 c.c.) | +++ | ++ | +++ | ++ | + |
| 42 (0.1 c.c.) | +++ | +++ | ++ | + | |
| 96 (0.1 c.c.) | +++ | +++ | ++ | + | − |
| I (0.1 c.c.) | + | + | − | − | − |
| II (0.1 c.c.) | + | ± | − | − | − |
| 49 (0.1 c.c.) | − | − | − | − | − |
| 174 (0.1 c.c.) | − | − | − | − | − |

TABLE IV.

*Serum 68 in Diminishing Quantities with Constant Amount (0.1 Cubic Centimeter) of Antigen.*

| Antigen No. | 0.005 c.c. | 0.0025 c.c. | 0.0012 c.c. | 0.0006 c.c. | 0.0003 c.c. |
|---|---|---|---|---|---|
| 68 (0.1 c.c.) | +++ | +++ | ++ | + | + |
| 19 (0.1 c.c.) | ++ | ++ | ± | − | − |
| 26 (0.1 c.c.) | +++ | +++ | ++ | + | − |
| 42 (0.1 c.c.) | +++ | +++ | ++ | + | ± |
| 54 (0.1 c.c.) | +++ | +++ | ++ | + | − |
| 96 (0.1 c.c.) | +++ | +++ | ++ | ++ | + |
| I (0.1 c.c.) | ++ | + | − | − | − |
| II (0.1 c.c.) | + | ± | − | − | − |
| 49 (0.1 c.c.) | − | − | − | − | − |
| 174 (0.1 c.c.) | − | − | − | − | − |

TABLE V.

*Serum 96 in Diminishing Quantities with Constant Amount (0.1 Cubic Centimeter) of Antigen.*

| Antigen No. | 0.005 c.c. | 0.0025 c.c. | 0.0012 c.c. | .0006 c.c. | 0.0003 c.c. |
|---|---|---|---|---|---|
| 96 (0.1 c.c.) | +++ | +++ | +++ | +++ | +++ |
| 19 (0.1 c.c.) | +++ | +++ | ++ | +± | − |
| 26 (0.1 c.c.) | +++ | +++ | +++ | +++ | +++ |
| 42 (0.1 c.c.) | +++ | +++ | +++ | +++ | ++ |
| 54 (0.1 c.c.) | +++ | +++ | +++ | +++ | ++ |
| 68 (0.1 c.c.) | +++ | +++ | ++ | + | + |
| I (0.1 c.c.) | ++ | ++ | − | − | − |
| II (0.1 c.c.) | + | ± | − | − | − |
| 49 (0.1 c.c.) | − | − | − | − | − |
| 174 (0.1 c.c.) | − | − | − | − | − |

TABLE VI.

*Serum I in Diminishing Quantities with Constant Amount (0.1 Cubic Centimeter) of Antigen.*

| Antigen No. | 0.05 c.c. | 0.01 c.c. | 0.005 c.c. | 0.0025 c.c. |
|---|---|---|---|---|
| I | +++ | +++ | ++ | + |
| II | + | + | − | − |
| 19 | ++ | ++ | + | − |
| 26 | +++ | ++ | + | − |
| 42 | +++ | +++ | ++ | + |
| 68 | ++ | ++ | + | − |
| 96 | +++ | +++ | ++ | − |
| 49 | − | − | − | − |
| 174 | − | − | − | − |

TABLE VII.

*Serum II in Diminishing Quantities with Constant Amount (0.1 Cubic Centimeter) of Antigen.*

| Antigen No. | 0.05 c.c. | 0.01 c.c. | 0.005 c.c. | 0.0025 c.c. |
|---|---|---|---|---|
| II (0.1 c.c.) | +++ | ++ | + | − |
| I (0.1 c.c.) | ++ | + | − | − |
| 19 (0.1 c.c.) | ++ | + | − | − |
| 26 (0.1 c.c.) | ++ | + | − | − |
| 42 (0.1 c.c.) | ++ | ++ | − | − |
| 68 (0.1 c.c.) | ++ | + | − | − |
| 96 (0.1 c.c.) | ++ | + | − | − |
| 49 (0.1 c.c.) | − | − | − | − |
| 174 (0.1 c.c.) | − | − | − | − |

TABLE VIII.

*Serum 26, 0.1 Cubic Centimeter, with Decreasing Amounts of Antigen.*

| Antigen No. | 0.05 c.c. | 0.04 c.c. | 0.03 c.c. | 0.02 c.c. | 0.01 c.c. |
|---|---|---|---|---|---|
| 26 | +++ | +++ | +++ | ++ | − |
| 19 | +++ | ++ | + | ± | − |
| 42 | +++ | +++ | ++ | + | + |
| 54 | +++ | +++ | ++ | + | − |
| 68 | +++ | +++ | +++ | + | − |
| 96 | +++ | ++ | ++ | + | − |
| I | ++ | + | − | − | − |
| II | + | − | − | − | − |

TABLE IX.

*Serum 42, 0.1 Cubic Centimeter, with Decreasing Amounts of Antigen.*

| Antigen No. | 0.05 c.c. | 0.04 c.c. | 0.03 c.c. | 0.02 c.c. | 0.01 c.c. |
|---|---|---|---|---|---|
| 42 | +++ | +++ | ++ | + | + |
| 19 | ++ | ++ | + | − | − |
| 26 | +++ | +++ | ++ | + | − |
| 54 | +++ | +++ | + | ± | − |
| 68 | +++ | +++ | ++ | + | + |
| 96 | +++ | +++ | ++ | + | − |
| I | ++ | ++ | − | − | − |
| II | + | − | − | − | − |

TABLE X.

*Serum 96, 0.1 Cubic Centimeter, with Decreasing Amounts of Antigen.*

| Antigen No. | 0.05 c.c. | 0.04 c.c. | 0.03 c.c. | 0.02 c.c. | 0.01 c.c. |
|---|---|---|---|---|---|
| 96 | +++ | +++ | +++ | ++ | + |
| 19 | +++ | +++ | ++ | + | − |
| 26 | +++ | +++ | +++ | ++ | + |
| 42 | +++ | +++ | +++ | ++ | + |
| 68 | +++ | +++ | +++ | ++ | + |
| I | ++ | ++ | ++ | + | − |
| II | + | + | − | − | − |

As shown in the tables, there has been a certain amount of cross-fixation, or group reaction, among the pneumococci. This group reaction is most clearly demonstrated by using immune sera I and II with *Pneumococcus mucosus* antigens (tables VI and VII). Antigen I has constantly reacted more strongly with *Pneumococcus mucosus* sera than has antigen II. There can be no doubt that, as shown by Dochez, these two organisms are essentially different; they likewise differ in essential points from *Pneumococcus mucosus.*

No cross-fixation was observed in testing the complement-fixing

powers of sera 49 (*Streptococcus mucosus*) and 174 (*Streptococcus pyogenes*). They each fixed specifically with homologous antigens, but showed no fixation with heterologous antigens.

From the foregoing considerations, the conclusion is justifiable that Schottmüller's *Streptococcus mucosus* is in reality a variety of pneumococcus. Its cultural characteristics, solubility in solutions of bile salts, and finally its behavior in comparative complement fixation experiments all support this conclusion.

### PROTECTION EXPERIMENTS.

The homogeneous character of the *Pneumococcus mucosus* group would, of course, be a most fortunate condition if an antiserum capable of conferring passive immunity could be produced. To determine the possibility of obtaining such a serum has been one of the aims of this study. A high grade of active immunity to *Pneumococcus mucosus* has been produced in rabbits, and the antisera obtained have been used in attempting to protect white mice against this organism. Immune serum and the bouillon culture of the organism were injected simultaneously into the peritoneal cavity. The most favorable result obtained is shown in the following protocol:

| Organism 42. | Antiserum 42. | |
|---|---|---|
| 0.000001 c.c. | 0.5 c.c. | Dead in 60 hours. |
| 0.00001 c.c. | 0.5 c.c. | Dead in 35 hours. |
| 0.0001 c.c. | 0.5 c.c. | Dead in 18 hours. |
| Control. | | |
| 0.000001 c.c. | | Dead in 18 hours. |
| 0.00001 c.c. | | Dead in 15 hours. |
| 0.0001 c.c. | | Dead in 14 hours. |

A slight prolongation of life would seem to have resulted from the use of 0.5 of a cubic centimeter of antiserum, but this result has not been constant with all the sera employed. As a rule the experimental animals and the controls have died within approximately the same lengths of time.

We are confronted, then, with the curious fact that the sera of animals possessing a high degree of active immunity fail to confer passive immunity to homologous organisms upon other animals.

This result is the more surprising because of the relative ease with which protecting antisera can be obtained with other strains of pneumococci. For example, rabbits which have received ten cubic centimeters of a twenty-four hour bouillon culture of pneumococcus I as the maximum immunizing dose, furnish sera which will protect against 0.1 of a cubic centimeter of this organism, whereas the sera of rabbits which have received 100 cubic centimeters of *Pneumococcus mucosus* as a maximum dose fail to protect mice against 0.000001 of a cubic centimeter of the homologous organism. These facts indicate the existence of fundamental differences between *Pneumococcus mucosus* and other varieties of pneumococci, and it is of great interest to inquire into the causes which may possibly underlie these differences.

The failure of *Pneumococcus mucosus* antisera to protect is due in all probability to some peculiarity in the constitution of this organism rendering it extremely resistant to the action of specific antisera, and one naturally thinks first of the heavy mucoid capsule. It is instructive to recall in this connection certain facts which have come to light in the study of the encapsulated bacteria. With these organisms, as with *Pneumococcus mucosus,* no protection has been obtained, although active immunity is produced without difficulty (9). Porges (5) cites an interesting observation upon *Bacillus pneumoniæ* of Friedländer. A strain of this organism which had been cultivated for a long period upon artificial media showed no capsule formation and could be agglutinated without previous treatment. It thus seemed a very favorable organism with which to test for specific protection, but when injected into animals together with specific antiserum, no protection was afforded and it was found that the organisms had developed capsules in the animal body. The somewhat similar experiences of Gruber (10) and Löhlein (11) with anthrax and plague bacilli likewise seem to indicate that the serum resistance of certain organisms may be due to capsule formation. As to the correctness of this, judgment must be suspended until more facts are at hand. The problem seems worthy of more careful study than it has as yet received.

Lobar pneumonia caused by *Pneumococcus mucosus* has been in the experience of this hospital a very fatal disease, six of our nine

cases (66 per cent.) having died. The organism was cultivated from the blood in four of the nine cases and none of these recovered.

## CONCLUSIONS.

1. The organisms described by Schottmüller under the name *Streptococcus mucosus* represent a well defined group with characteristics which indicate a closer relationship to the pneumococci than to the streptococci.

2. The members of this group are specifically agglutinable when treated according to the method of Porges. They do not agglutinate when subjected to the usual agglutination methods.

3. Complement fixation experiments with these organisms, compared with similar experiments with two varieties of pneumococci and two streptococci, indicate that they are closely related to the pneumococci.

4. No protection of mice against *Pneumococcus mucosus* by means of specific immune sera could be demonstrated.

5. The name *Pneumococcus mucosus* should be adopted for this group instead of *Streptococcus mucosus.*

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# ON THE CAUSATION BY FILTERABLE AGENTS OF THREE DISTINCT CHICKEN TUMORS.*

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Plates 7 to 12.

The study of chicken tumors has especial interest because the cause of two such growths has been found in filterable entities. The object of the present paper is to report experiments which show that yet a third neoplasm of the fowl is so caused, and to discuss at some length the methods and findings with all three. One of the growths, —Chicken Tumor I in our series of spontaneous chicken tumors,— is a pure spindle-celled sarcoma;[1] the second (Chicken Tumor VII) is an osteochondrosarcoma;[2] and the third (Chicken Tumor XVIII), of which the cause will here for the first time be reported, is a spindle-celled sarcoma of peculiar intracanalicular pattern.[3] The three are very unlike, not only histologically but in their general behavior. Yet, as will be seen, the entities causing them have much in common and may profitably be considered together.

## THE IMPORTANCE OF PRELIMINARY TRANSPLANTATION.

In our experience the transplantation of chicken tumors is of great importance for experiments looking to their cause. This is in part on account of the material afforded by successful transplantation, but it has its essential basis in the enhanced malignancy resulting therefrom. The findings have shown strikingly that the more malignant the growth the easier is the demonstration of its etiological agent. The fact that tumors caused by an agent dam-

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[1] Rous, P., *Jour. Am. Med. Assn.*, 1911, lvi, 198; *Jour. Exper. Med.*, 1911, xiii, 397. For the literature see Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, 1913, l, 637.

[2] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lix, 1793. Tytler, W. H., *Jour. Exper. Med.*, 1913, xvii, 466.

[3] Rous, P., and Lange, L. B., *Jour. Exper. Med.*, 1913, xviii, 651.

aged in some way, as for example by heat or by long sojourn in the dry state, are of relatively benign course[4] suggests that the ready separation of the agent from growths of high malignancy is dependent on its enhanced activity.[5]

### THE TUMOR-PRODUCING ENTITIES ARE FILTERABLE.

Three methods, namely filtration, desiccation, and glycerination, have been chiefly used to demonstrate a cause for the chicken tumors, as distinct from the tumor cells. Specimen protocols will be given to illustrate the results with each method and these will be followed by a summary of the findings obtained by its employment. The growths produced have in every instance been examined with the microscope.

Filtration has proved by far the most uniformly successful method of study. Under suitable conditions the agents of all the growths will pass through Berkefeld filters that retain small bacteria (*Bacillus prodigiosus, Bacillus fluorescens liquefaciens*). Our technique has been described several times. It is again briefly given in the first of the two following protocols that show the filterability of the agent causing Chicken Tumor XVIII.

*Chicken Tumor XVIII, a Sarcoma of Intracanalicular Pattern. Filtration Experiment 1.*—The tumor material came from fowl 257 of the second transplantation generation, series A. Fresh neoplastic tissue to the amount of 7.5 gm. was ground fine with sand, taken up in 250 c.c. of Ringer's solution at 40° C. and a 24 hour culture on slant agar of *Bacillus fluorescens liquefaciens* was added. Shaking was done in a machine for forty-five minutes, then brief centrifugalization, and portions of the supernatant fluid were filtered by suction through one or another of three Berkefeld filters; (a) a medium sized V (No. 3), and (b) and (c) two small N cylinders (No. 5). Filtration was continued for about thirty minutes. The fluids from filters a and b were united as filtrate AB. That from filter c will be called filtrate C. To each was added a little sterile diatomaceous earth (*Kieselguhr*) and then 14 c.c. of AB and 7 c.c. of C were injected intramuscularly in the right and left pectoral regions respectively of five normal brown Leghorn fowls. All remained free of tumor until 170 days after the inoculation, when in one a small mass, 2.5 by 1.5 cm. in diameter was noted at the site of injection of filtrate AB. Part of this was removed and found to have

[4] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1913, xvii, 219.

[5] It may not be amiss to point out that in epidemic poliomyelitis, a disease caused by a filterable virus or parasite, the initial infection of monkeys with human nervous tissues is accomplished with difficulty, while after the virus has

the characteristic structure of Chicken Tumor XVIII. The fowl was shortly afterwards lost through accident. The other chickens were kept some months longer but never developed tumors.

Plates of the filtrates (1 c.c. to the tube of agar) remained sterile, whereas plates of the unfiltered fluid (a few drops to the tube of agar) showed innumerable colonies of *Bacillus fluorescens liquefaciens.* At the time of the experiment bits of the tumor which furnished material for it were transplanted into three chickens. Two of these developed tumors within a month. The third remained healthy.

*Filtration Experiment 2.*—The material was furnished by fowl 531 of the third transplantation generation, series C. The tumor was of especially rapid growth. Three Berkefeld filters were employed: (a) a medium sized V cylinder (No. 3), and (b) and (c) two small N cylinders (No. 5). *Bacillus fluorescens liquefaciens* was used as before; and the filters proved impermeable to it. To each of the filtrates, A, B, and C, a little finely ground, sterile, diatomaceous earth was added previous to their separate injection in amounts of 5 c.c. each, into the muscle of the lower leg and breast of eight fowls. Two of the fowls developed progressively growing tumors which appeared, in the one case at sites A and C two months after the time of injection, in the other at sites A and C about five months after it. Both died of the tumor some seven and one half months after inoculation. In addition to the large primary growths there were metastases in the lungs, and in one case secondary growths about several joints (figure 1), a finding not infrequent when Chicken Tumor XVIII is propagated by transplantation. The hip joint was diffusely involved in sarcomatous tissue and fusiform swellings enclosed most of the sternal and vertebral ribs at their junction, forming what might be called a sarcomatous rosary. Whether in the present case these represent primary localizations of the tumor-producing virus or true metastases cannot be said. Microscopically all the growths had the characteristic structure of Chicken Tumor XVIII (figure 2).

In a third fowl a tumor nodule appeared within two months at the site of filtrate B, slowly enlarged to a diameter of 4 cm., and slowly retrogressed again. The only transplantations attempted from the filtrate tumors were performed with this growth. They gave negative results. The other fowls injected with filtrate remained healthy.

The association of a foreign body with the filtrate to bring about a tissue derangement renders much more likely the production of tumors. The influence of the factor has been carefully studied in the case of Chicken Tumor I.[6] In all the experiments we have made use of powdered diatomaceous earth (*Kieselguhr*), which elicits, as Podwyssozki has shown, an intense reactive connective tissue proliferation with the formation of giant cells. Histologically this

been transmitted through several monkeys, it increases markedly in virulence for those animals and can be transferred with far greater certainty.

[6] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

reactive proliferation bears no resemblance to the tumors with which we have worked (figure 10).

*Chicken Tumor XVIII. Filtration Experiment 3.*—Filtrates were prepared in the usual way, through filters which the control with *Bacillus fluorescens liquefaciens* showed to be bacteria-tight. Three Berkefeld cylinders were used: (a) a large unmarked cylinder (No. 2), (b) a medium V (No. 3), and (c) a small N (No. 5). The filtrates were separately examined for the test bacterium, and injected. To half of each was added enough sterile, powdered *Kieselguhr* to give a slight cloudiness. For this very little was required. Six fowls were injected, receiving on one side in the thigh and pectoral muscle respectively two filtrates with *Kieselguhr,* at corresponding points on the other side the same filtrates in equal or slightly greater amount, without *Kieselguhr.* Of the five fowls that survived three months one developed after the sixtieth day small tumor nodules, where the filtrates A and C had been injected in amounts of 10 c.c. and 3 c.c. respectively with *Kieselguhr.* The corresponding sites, where slightly larger quantities of the filtrates without admixture had been put, remained free of growths. The tumors measured 4 cm. and 3 cm. in diameter respectively when the fowl was killed three months after injection. They had the characters of Chicken Tumor XVIII. The other fowls, kept long under observation, remained healthy.

*Chicken Tumor VII, an Osteochondrosarcoma. Filtration Experiment 2.*—Six normal fowls were injected in the pectoral muscle on the right side with 6 c.c. each of an active Berkefeld filtrate, in the right thigh muscles with 4 c.c. of the same filtrate to which had been added a little *Kieselguhr.* It has been repeatedly found that the thigh muscles furnish a relatively poor site of inoculation. Nevertheless, in four of the fowls rapidly growing cartilaginous tumors of multicentric origin appeared in the thigh (figures 3 to 8), whereas a pectoral tumor developed in only two of the four. It took the form of a discrete cartilaginous nodule in the track of the injecting needle. The other two fowls remained healthy. One of the tumor fowls furnished material for the experiment which follows and also for a successful attempt to dry the etiological agent. Some of the growths contained so much bone that the saw was required to lay them open.

*Filtration Experiment 4.*—Portions of a tumor extract prepared as usual and with the usual bacterial control were passed through one of the following Berkefeld cylinders: (a) and (b) two small N cylinders (No. 5), and (c) a medium sized V (No. 3). The tumor material came from one of the fowls of filtration experiment 2. Filtrates A and B were injected separately in amounts of 10 c.c. into the leg muscles of three normal fowls. Filtrate C with and without admixture of *Kieselguhr* was injected into the pectoral regions of the same fowls. Nineteen days later one had developed a tumor nodule 1.3 cm. in diameter at the spot where 5 c.c. of filtrate C with *Kieselguhr* had been injected. The nodule rapidly enlarged to a diffuse mass which led to the death of the fowl after six weeks in all. At this time a nodule had just appeared in the other pectoral region where 10 c.c. of filtrate C without admixture had been put. At autopsy another small discrete nodule was found in the left leg as the result of 10 c.c. of filtrate A without *Kieselguhr.* The other two fowls remained healthy.

*Chicken Tumor 1, a Spindle-Celled Sarcoma. Injury Experiment 15.*—An active Berkefeld filtrate was prepared as usual and to half of it a little *Kieselguhr* was added. 9 c.c. of the mixture was injected into the pectoral muscles on one side of eight fowls, and on the other as control an equal amount of the plain filtrate. The results are shown in text-figure 1. It will be seen that where there was *Kieselguhr* tumors arose much more rapidly, and when palpable were already diffuse. In this instance nearly all the fowls responding with a tumor to the filtrate plus *Kieselguhr* eventually developed growths where the filtrate alone had been placed. Ordinarily this happens in only a small percentage.

SUMMARY ON FILTRATION.

The growths engendered by a Berkefeld filtrate have the distinctive characters of the strain of tumor which furnished the material for filtration (figures 1 to 9). In the case of Chicken Tumors I and VII growths so caused have themselves been successfully used for the preparation of filtrates, as for example in one of the experiments just cited. They have also been found to be transplantable. The results with Chicken Tumor XVIII are relatively meagre owing to the long period of latency after the injections,—more than five months in one case,—and to the slow growth of the filtrate tumors. The agents causing Chicken Tumors I and VII often pass the filter in large amount, as shown by the multicentric development of tumors in the region injected, but even when the element of tissue derangement is present, Chicken Tumor XVIII usually appears from one, or at most a few, centers. This is more probably due to low average virulence or resistance in the agent than to difficulties in filtration, for the findings show that all the agents pass or are held back by filters of about the same texture. They pass most V cylinders, many designated as N, but they are usually retained by the fine textured W cylinders. As might be expected from these results the agent of Chicken Tumor I,—the only one thus tested so far—fails to pass Chamberland bougies.

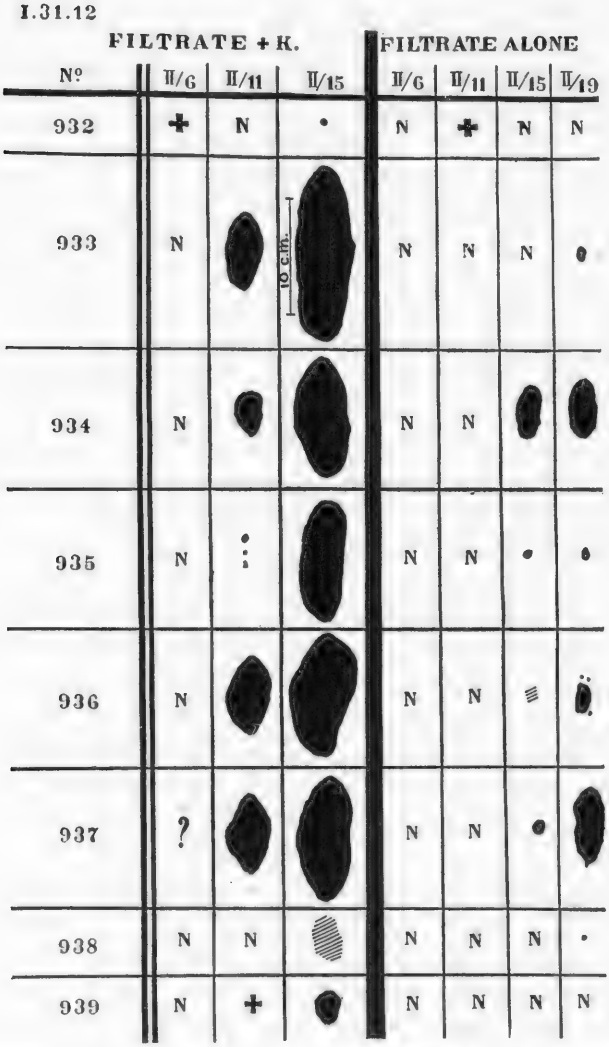
Not infrequently filtrates prepared from malignant material under the best conditions prove entirely innocuous. This is in most cases due to the narrow limits within which the agents are filterable. Two possibilities suggest themselves as accounting for these limits, first that the agents are formed bodies, second that if unformed they are associated with substances which clog the pores of the filters. Mucinous substances are so abundant in Chicken Tumor I

that coarse filters are soon completely stopped by very dilute extracts of the growth; but with VII and XVIII there is no such complication. Extracts of these tumors run rapidly through the filters and yet are often inactive. The agents then would seem to be of relatively large size among the filterable causes of disease.

The tumor resulting from a local injection of filtrate appears at the site of inoculation. As already mentioned the importance of tissue derangement for the action of all three causative agents is very great. The experiments show that when this factor is supplied by the addition of *Kieselguhr* to a filtrate the percentage of fowls that develop tumors is much increased and the growths themselves appear sooner and enlarge more rapidly (text-figure 1). The *Kieselguhr,* injected alone, does not cause tumors. A limpid filtrate injected in the breast muscle finds its point of action in the track of the injecting needle (figure 9), and there results a discrete growth from one center. Oftentimes, as ordinarily prepared, the filtrate contains a few particles from the interior of the filter, and the growth may then arise from several centers. But when powdered *Kieselguhr* has been added the growth is multicentric and appears all at once as a mass of coalescing foci. The microscopic findings show that the sarcomatous change in the reactive tissue about the *Kieselguhr* is not diffuse but punctate, and the growth of the little tumors is largely expansive (figure 10). In fowls inoculated intravenously with a filtrate of Chicken Tumor I the tumors have been found to arise at sites of tissue derangement.[7] Their incidence is approximately doubled when *Kieselguhr* has been introduced into the blood stream.

As already mentioned the reactive tissue called forth by *Kieselguhr* contains large numbers of giant cells. These are of ordinary foreign body type and enclose the *Kieselguhr* fragments. With the replacement of the reactive tissue by tumor the giant cells are destroyed (figure 10) and the *Kieselguhr* is set free. It can be found here and there among the sarcoma cells, which appear in no way affected by it.

[7] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.,* 1912, lviii, 1751.




TEXT-FIG. 1. CHICKEN TUMOR I. INJURY EXPERIMENT 15. Eight normal Plymouth Rock fowls, Nos. 932 to 939, were injected in one pectoral region with a Berkefeld filtrate of Chicken Tumor I, in the other with an equal quantity of the same filtrate to which had been added a little powdered diatomaceous earth (*Kieselguhr*). Examinations were made every week thereafter and the size of the tumors charted. It will be seen that where filtrate plus *Kieselguhr* was injected tumors appeared more frequently and much earlier, and were diffuse when first noted in sharp contrast to the discrete nodules on the other side.

DESICCATION.

Since many microörganisms withstand drying and the tissue cells of the higher animals do not, workers have frequently used desiccation in attempts to separate an etiological agent from mammalian tumors. The literature need not be discussed since it deals only with negative results. The agents causing two of our chicken tumors, namely the pure spindle-celled sarcoma (Chicken Tumor I), and the osteochondrosarcoma (Chicken Tumor VII), resist drying. No special complications are met with in obtaining an active dry form of the agent of Chicken Tumor I, but in the case of Chicken Tumor VII successful results have been obtained only by the desiccation of frozen material. A few specimen protocols will be given.

*Chicken Tumor I. Desiccation Experiment 2.*—The tumor material, obtained from fowl 243 of the ninth transplantation generation, series A, was ground fine and spread thin in a desiccator containing sulphuric acid. A partial vacuum only was produced. After forty-eight hours the scales of dry tissue were pulverized in a mortar and replaced in the desiccator. After sixty-seven hours in all the powder was taken up in Ringer's solution by grinding, in the proportion of 2 gm. to 70 c.c. 5 c.c. of the turbid, viscous fluid were injected into each breast of seven normal hens. Six showed rapidly growing tumors at the end of the month. The seventh remained healthy. One of the fowls furnished tumor material for the experiment which follows.

*Desiccation Experiment 4.*—A fowl of experiment 2, with a large tumor, was killed and the neoplastic material dried as before, for forty-eight hours; ground; replaced in the desiccator for seventy-two hours more; and then sealed in glass and kept in the dark at about 2° C. Fifty-four days later 2 gm. of the material were taken up in 25 c.c. of Ringer's solution, and the thick suspension was inoculated in amounts of 5 c.c. into each breast of five normal hens. Three of these had developed tumors two weeks later, and one growth was already large. In a fourth fowl the tumor appeared later. The fifth remained quite healthy.

Some of the dried material was left sealed and in the cold for seven months. 3 gm. of it were then taken up in 20 c.c. of normal salt solution and 1 c.c. of the mixture was injected into one breast of eight fowls. Of these two had developed small tumors after forty days. The others remained healthy. The growths were typical Chicken Tumor I.

*Chicken Tumor VII. Desiccation Experiment 3.*—Fowl 549, carrying a large growth engendered by a filtrate (filtration experiment 2) was killed, and the tumor, which consisted of cartilage and precartilaginous, sarcomatous tissue, was ground fine and placed in a cold compartment at several degrees below 0° C., where in the course of forty-eight hours it gradually froze solid. While still frozen it was put in a cold desiccator over sulphuric acid, and the air exhausted until the manometer showed a pressure of less than 1 mm. of mercury. The desiccator was then placed immediately in an ice chest. At the end of three days the material was taken out and ground. It had the form of a light, brittle cake

of nearly the bulk of the fresh tissue, was cottony in texture, and ground with great difficulty. Twenty-four hours after grinding, 2 gm. of the powder were taken up in 25 c.c. of sterile water and of the thick fluid 5 c.c. were injected into the left breast of each of five normal fowls. Within a month one developed a growth measuring 4 by 9 cm. It was killed and portions of the tumor were used for the experiment that follows. The tumor consisted of a spindle-celled sarcomatous tissue undergoing cartilaginous differentiation in the way characteristic of Chicken Tumor VII. The other fowls, kept under observation several months, remained healthy.

*Desiccation Experiment 4.*—The material came from the susceptible fowl of the previous experiment. It was ground, frozen, and dried while still frozen, by the method just outlined. It remained in the freezing chamber three days, in the desiccator twenty-four hours, and was then powdered and placed in the ice chest for twenty-four hours more. Eight fowls were injected in the left breast with 5 c.c. each of a thick fluid made by taking up 2 gm. of the powder in 40 c.c. of distilled water. In the other breast glycerinized tumor tissue was placed at the same time (glycerin experiment 2). Within three weeks all except one of the injected fowls had developed a tumor mass at the site of inoculation of the dried material, the largest measuring 3.4 by 7.5 cm. Four of the fowls were allowed to die of their growth, the other three being killed and examined at various times. All the growths presented the character of Chicken Tumor VII, with much cartilage and in several instances bone. The eighth fowl remained healthy.

In this experiment there was the complicating factor of tumors developing synchronously from glycerinated material. But that the tumors which arose where dried material had been injected were engendered by this is certain, not only from the local character of the neoplastic disease in its early stages, but from many other instances that might be cited in which the dried tissue of Chicken Tumor VII has been successfully used to cause the growth.

### SUMMARY ON DESICCATION.

The growths caused by dried tissue of Chicken Tumors I and VII appear at the site of injection and themselves furnish material which has a tumor-producing activity when dried. The etiological agent of Chicken Tumor I undergoes a gradual attenuation when the dried tissue is stored in the dark at a temperature slightly above 0° C., but after seven months is still capable of producing tumors. In the case of Chicken Tumor VII no attempt has been made to store dried material for longer than three weeks. At the end of this time it still produced tumors.

Numerous attempts have been made to obtain the agent of Chicken

Tumor XVIII in an active dry form, but as yet without success. In this connection it should be recalled that Chicken Tumor XVIII under the best conditions grows much more slowly than I and VII and that a filtrate of it gives rise only exceptionally to tumors and after a long latent period. The agents of Chicken Tumors I and VII when dried at low temperature are both very active; but the one easily survives drying at room temperature whereas the other fails to. Differences in the viability of organisms would well account for this.

The growths caused by dried material spring from multiple foci and are often diffuse when first palpable. The bits of dead tissue doubtless act to produce like *Kieselguhr* a tissue derangement favorable to the production of tumors.

GLYCERINATION.

The effect of glycerin has especial interest because of the ultramicroscopic organisms which retain their vitality when immersed in it. Loeb[8] has observed its effect upon mammalian tumor tissue. He placed pieces of rat sarcoma in pure glycerin, and, seventeen to twenty-four hours later, washed them in salt solution and injected bits into other rats. In some instances a tumor developed. If it can be assumed that the glycerin thoroughly penetrated the tumor tissue the finding is significant. For this reason we have repeated and enlarged upon Loeb's experiment, previous to observations with the chicken tumors. Briefly, the findings show that pieces of the Jensen rat sarcoma 0.5 cm. thick, when kept for twenty-four hours in pure glycerin at a temperature of 1° to 2° C., will sometimes give rise to tumors on being cut up and implanted; but the same material, finely chopped and stirred into the glycerin, yields only negative results, even when well washed and injected in quantity. Rat sarcoma treated according to the latter method occasionally retains its viability for twenty-four hours in a 12.5 or 25 per cent. glycerin mixture with Ringer's solution, but never in 50 per cent. glycerin, nor for a longer period (four days) in the weak dilutions mentioned. Hence it seems then that the positive results with pieces of rat tumor kept in concentrated glycerin are dependent on incomplete penetration.

[8] Loeb, L., *Virchows Arch. f. path. Anat.*, 1903, clxxii, 345.

Of the filterable entities that cause chicken tumors the two which withstand drying will also survive glycerination.

*Chicken Tumor I. Glycerin Experiment 3.*—13 gm. of fresh tumor tissue from fowl 582 (16th generation B) were ground with sterile sand, and suspended in 40 c.c. of Ringer's solution. The larger tissue particles were got rid of by straining, and the sand was allowed to settle out. Of the thick suspension of tissue 10 c.c. were stirred into an equal amount of pure glycerin, shaken until well mixed and placed in the ice box. After seven days the tissue fragments, which had by this time settled, were drawn off through the base of the tube by breaking a sterile glass projection in its wall. In this way all contamination with particles which had escaped the glycerin's action was avoided. The tissue suspension was now washed with Ringer's solution, centrifugalized, and of the pasty sediment 0.15 c.c. was injected into the right and left pectoral muscles respectively of three young Plymouth Rock fowls. Ten days later in one fowl a small sarcomatous nodule made its appearance at each injection site, and in a second after seventeen days a single nodule developed. The subsequent growth of the tumors was slow, but otherwise they proved characteristic of Chicken Tumor I. The third fowl remained healthy.

*Glycerin Experiment 2.*—Fresh tumor tissue was ground in a mortar with sand and taken up in Ringer's solution. By rapid centrifugalization the suspension was rid of all but extremely minute tumor fragments. To portions of it pure glycerin was added to the amount of 50, 25, and 12 per cent. respectively of the total bulk. The mixtures were placed in wide tubes and kept in the dark at 1° to 2° C. After nine days some of the material was withdrawn by breaking a projection at the base of the tube, as in the experiment already described. Twenty-two days later a fowl injected in each pectoral region with 0.4 c.c. of the mixture in 50 per cent. glycerin, diluted to 4 c.c. with Ringer's fluid, had developed very large tumors having the character of Chicken Tumor I. In the three fowls injected with 12 and 25 per cent. mixtures, variously diluted, growths had also appeared at this time.

Some tubes of the 12.5 and 25 per cent. mixtures were left untouched and in the cold for thirty-one days. Their contents were then drawn off as usual. One fowl injected in each breast with 3 c.c. of 25 per cent. mixture, undiluted, developed tumors. These first became palpable after six weeks. Another fowl injected in the same way with 1 c.c. of the 25 per cent. mixture made up to 2 c.c. with Ringer's fluid likewise slowly developed tumors. Three fowls inoculated with the 12.5 per cent. mixture, diluted with an equal bulk of Ringer's solution, remained healthy.

Higher percentages of glycerin have been used in the experiments with Chicken Tumor VII.

*Chicken Tumor VII. Glycerin Experiment 3.*—Cartilaginous tumor material was ground to a foamy pulp in a meat chopper and two mixtures were made with pure glycerin, in the amounts of (a) 2 c.c. tumor pulp and 8 c.c. glycerin, and (b) 4 c.c. tumor pulp and 10 c.c. glycerin. The actual quantities of tumor tissue were considerably less than those mentioned, as it had the form of a

soufflé. The mixtures were tubed and kept in the cold as usual. Sedimentation in the glycerin was extremely slow and shaking was done each day to keep the larger tissue fragments in suspension. After thirteen days the tubes were opened at the base, and the mixtures, made up to 20 c.c. with Ringer's solution, were injected in amounts of 5 c.c. into the right and left pectoral region, respectively, of three normal fowls. After two months one fowl developed a tumor that in another three months proved fatal. The growth was 6 cm. in diameter at this time and in addition to much cartilage contained bone. It had arisen at the site of injection of the mixture that contained the greater percentage of glycerin (8 c.c. to 2 c.c. of tumor). The other fowls remained healthy.

*Glycerin Experiment 4.*—Fresh cartilaginous tumor material was ground to a soufflé and three mixtures were made: (a) 10 c.c. of pure glycerin with an equal bulk of tumor material, (b) 10 c.c. of glycerin with 5 c.c. of tumor, and (c) 10 c.c. with 3 c.c. of tumor. These were kept in the ice box; shaken every day; at the end of ten days drawn off from below; and each made up to 20 c.c. with Ringer's solution and inoculated in amounts of 5 c.c. in one pectoral region of five normal fowls. Four of these died of intercurrent disease. Ninety-nine days after the injection the fifth had developed a tumor measuring 8 cm. at the inoculation site of mixture (c), and one of half this size where mixture (a) had been put. Both growths were characteristic of Chicken Tumor VII and both contained bone.

### SUMMARY ON GLYCERINATION.

The experiments leave no doubt that the tumor tissue was thoroughly penetrated by glycerin. The agent of Chicken Tumor I retains some activity for at least seven days in 50 per cent. and for thirty-one days in 25 per cent. glycerin. The effect upon it of higher concentrations has not been tested. In the case of Chicken Tumor VII the amount of glycerin has ranged from 50 to about 90 per cent., but no attempt has been made to determine the period of survival of the agent. That it remains active in the high concentrations for at least thirteen days is shown by experiment 3. Glycerination undoubtedly has an attenuating action on both agents. The tumors develop in few hosts, and after a relatively long latent period, and often grow slowly. It has been repeatedly noted that the activity of the agents is best retained in high concentrations of glycerin. This might be thought due to differences in penetration of the tumor tissue attendant upon differences in concentration, were it not for the findings with rat sarcoma. Here concentrated glycerin proves the more injurious. Tissue autolysis in the dilute glycerin mixtures affords a better explanation of the results with the avian tumors. The most active agent, that of Chicken Tumor I,

has been found to be quickly destroyed by autolysis of the tumor tissue.

Repeated attempts to preserve in glycerin the agent causing Chicken Tumor XVIII have been unsuccessful.

### SEPARATION OF THE AGENTS BY OTHER METHODS.

Only in the case of Chicken Tumor I have still other attempts been made to distinguish the tumor-producing agent from the neoplastic cells. Ultraviolet rays kill the cells and leave the agent unharmed.[9] The method requires careful control and is not available for the separation of large amounts of the agent. The resistance of the agent to heat is only very slightly greater than that of the cells.[10] On the other hand, the agent withstands freezing and thawing which reduce the associated tumor tissue to a pulp.

### DISCUSSION.

In the first attempts to isolate a causative agent from Chicken Tumors VII and XVIII exact precautions were taken to avoid a possible contamination with the agent of Chicken Tumor I, though such contamination had never been observed in the many routine transplantations of these growths. To avoid exposure of the material to the laboratory air it was ground in a large sterile box and otherwise protected. The results of the experiments showed that these precautions were unnecessary, for the character of the tumors engendered by the agents effectually proved that they were not the result of contamination. Each agent produces only growths of the kind from which it came. One stimulates connective tissue to proliferate and elaborate cartilage, ultimately to be replaced in greater or less part by bone (Chicken Tumor VII); another causes connective tissue to proliferate and form large undifferentiated spindle-celled masses (Chicken Tumor I); while a third engenders, like the second, a spindle-celled growth, but one containing much collagen, and characteristically fissured by blood sinuses, into which the growth shows a tendency to extend, resulting in a complex intracanalicular pattern (Chicken Tumor XVIII). The behavior of the

[9] Rous, P., *Jour. Exper. Med.*, 1913, xviii, 416.

[10] Rous, P., and Murphy, J. B., *Jour. Am. Med. Assn.*, 1912, lviii, 1938.

several growths is as different as is their histology. Chicken Tumor I metastasizes to the lungs by preference, then to the further viscera; Chicken Tumor VII almost never gives metastases; and Chicken Tumor XVIII frequently disseminates to the muscles without other secondary localization. The individuality of the agents as exemplified in the neoplasms that they cause is not altered by attenuating them, yet it should be mentioned that Chicken Tumor XVIII, the fissured sarcoma, has recently shown a tendency, like some complex mouse tumors, to lose with repeated transplantation its histological peculiarities and become an undifferentiated spindle-celled sarcoma. Ultimately the agent causing it may produce tumors not very different from Chicken Tumor I.

The tumors engendered by the filterable agents become palpable only after a latent period,—which in the case of Chicken Tumor XVIII is usually several months. Chicken Tumors I and VII appear more promptly. Fowls which fail to develop the growths within one month after an injection of the agent remain free of them, as a rule. When large amounts of filtrate plus *Kieselguhr,* or of dried or glycerinated material, have been injected the resulting growths are diffuse when first observed, owing to proliferation from many foci, and they quickly become massive; but the period of latency—at least eight to ten days in the case of Chicken Tumors I and VII—is not appreciably shortened. That the greater part of this period is one of actual latency with tumor cells absent as yet, and is not merely an interval during which the tumor is growing but clinically imperceptible, has been determined by the early microscopic examination of sites where the agent is known to be present and active.

The injection of large amounts of the agent of Chicken Tumor VII results in tumors that grow progressively and soon lead to the death of the fowl; whereas the growths developing after implantation of a small bit of the tumor enlarge slowly and in most fowls become stationary and eventually retrogress. This difference might be thought due to the influence of dosage as affecting resistance,—a factor of much importance in the case of mouse tumors,—or it might conceivably result from the circumstance that growths induced by an agent are elaborated by the host's own tissues, while

such as result from transplantation represent tissue growing in a host to which it is strange. That the latter explanation is not fanciful has been shown by experiments with Chicken Tumor I demonstrating the existence in fowls of two types of resistance directed, the one against the tumor-producing agent, the other against the transplanted tumor cells.[11] But in the case now being considered differences in dosage as affecting resistance are probably at the root of the matter. For by the injection of large amounts of the fresh tissue of Chicken Tumor VII in the form of a pulp, progressively growing tumors, such as result from massive doses of the agent, can be obtained.

The growths resulting from injection of a tumor-producing agent into the skeletal muscles are at first purely local in character, even when a filtrate is employed. In only one among many autopsies have growths been found which by their situation suggested a possible primary dissemination by the blood stream. This case is cited in one of the protocols (Chicken Tumor XVIII, filtration experiment 2). There is, of course, no reason why part of a filtrate, injected with a sharp needle, should not often pass directly into some blood vessel. But it has been shown with Chicken Tumor I that the direct intravenous inoculation of an active filtrate usually fails to produce tumors.[12]

Many fowls are resistant to the tumor-producing agents in any of the forms that we have used. Glycerination markedly reduces the agents' activity and desiccation does so to a less degree, and roughly in proportion to the length of time that the material is kept after drying. Both filtrates and dried tissue prepared under the best conditions from malignant material are sometimes unaccountably inactive. More often the inactivity can be traced to the use of too concentrated extracts for filtration, too finely textured a filter, or too slow a process of drying.

The findings with the three tumor-producing agents have a striking similarity and it is difficult to avoid the conclusion that the three are of one class, whatever that class may be. All give rise to dis-

[11] Rous, P., *Jour. Exper. Med.*, 1913, xviii, 416.

[12] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

eases of neoplastic character, all act after a more or less pronounced latent period, the action of all depends to a striking extent on associated tissue derangement, and all pass through Berkefeld cylinders of about the same porosity, being held back by others of slightly finer grade. Two of them resist drying and can be preserved in glycerin. The third, which fails to retain its activity when so treated, causes tumors that are of relatively very slow growth (Chicken Tumor XVIII). Since in our experience the separation of a tumor-producing agent is largely a question of the growth's malignancy, it seems not improbable that with selective passage of the neoplasm an agent may eventually be obtained from it that is resistant to drying and glycerination.

The separation of etiological agents from three chicken tumors of such diverse character as those we have employed is strong evidence for the view that many other growths of the fowl have a like cause. It is hardly necessary to point out that were the latent period of Chicken Tumor XVIII, when produced by the specific agent, somewhat longer than the two to six months observed in the present investigation, or were the agent only very slightly more difficult to separate from the tissue by filtration, its presence would not have been demonstrated. Chicken Tumor XVIII would then have remained, with the sarcomata of the rat and mouse, among the transplantable tumors without a cause separable from tissue cells.

## CONCLUSION.

A causative agent has been separated from three chicken tumors of very different sort, namely a spindle-celled sarcoma, an osteochondrosarcoma, and a spindle-celled sarcoma peculiarly fissured by blood sinuses. This was accomplished after the tumors had been transplanted repeatedly and their malignancy enhanced. Each of the tumor-producing agents is a distinct entity in that it gives rise only to growths of the precise kind from which it has been derived. All pass through Berkefeld cylinders impermeable at the same test to small bacteria, and two of the three retain their activity in tumor tissue that has been dried or glycerinated. All are strikingly dependent for their action on derangement of the tissue with which they are brought in contact. The general findings strongly

suggest that the agents are of about the same size, and of the same natural class. It is perhaps not too much to say that their recognition points to the existence of a new group of entities which cause in chickens neoplasms of diverse character.

## EXPLANATION OF PLATES.[13]

### PLATE 7.

FIG. 1. Tumors caused by a Berkefeld filtrate of an extract of Chicken Tumor XVIII (a spindle-celled sarcoma fissured by blood sinuses) in Ringer's solution (filtration experiment 2). The sternum has been cut away and the body of the fowl eviscerated. In the left pectoral muscles is the large, pale, primary growth. On both sides at the junction of the sternal and vertebral ribs is the neoplastic rosary described in the text. That on the right has been cut through vertically. Its individual nodules have coalesced.

### PLATE 8.

FIG. 2. A section of one of the growths produced by a filtrate of Chicken Tumor XVIII (filtration experiment 2). The fissuring with blood channels, sometimes accompanied by intracanalicular growth, is characteristic of this tumor. The numerous black points in the channels are the nuclei of the red blood corpuscles.

FIG. 3. Large osteochondrosarcoma produced by the intramuscular injection of 4 c.c. of the Berkefeld filtrate of an extract of Chicken Tumor VII (filtration experiment 2). The fowl was killed when comatose, eighty-seven days after the injection. Its emaciation should be noted.

### PLATE 9.

FIG. 4. The growth shown in the preceding photograph, after it had been sawed open. Scattered amid the smooth, whitish cartilage is much bone with red marrow.

FIG. 5. An early stage of an osteochondrosarcoma produced by a filtrate of Chicken Tumor VII (filtration experiment 2). The fowl was killed when the tumor was first noted, eighteen days after injection. Here and there in the precartilaginous tissue, which has the general character of a spindle-celled sarcoma, the matrix of cartilage is in process of formation.

### PLATE 10.

FIG. 6. Another portion of the growth illustrated in figure 5. The formation of cartilage is well advanced.

FIG. 7. A section of the growth shown in figures 3 and 4. The cartilage is in process of replacement by bone. Note the calcification and the abundant red bone marrow.

[13] All the microscopic sections were stained with methylene-blue and eosin.

FIG. 1.

(Rous and Murphy: Causation of Chicken Tumors.

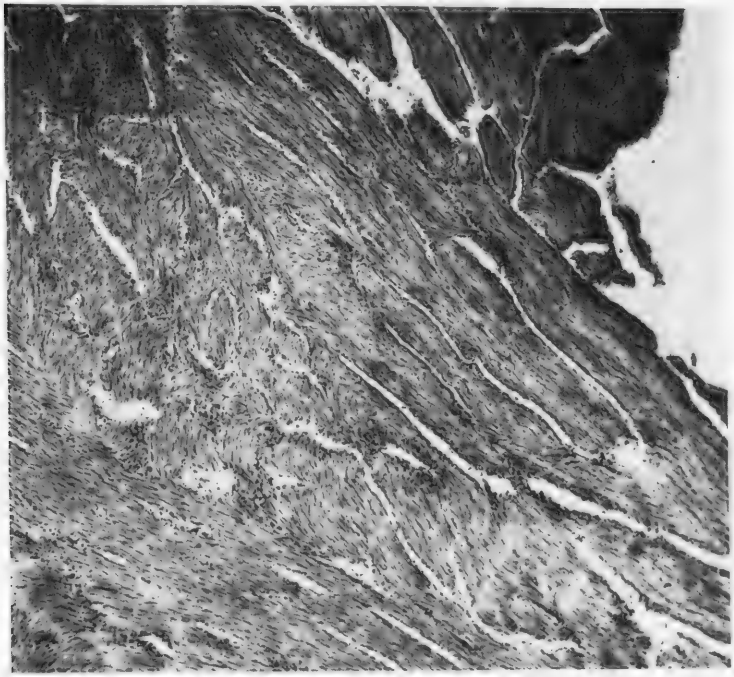


FIG. 2.

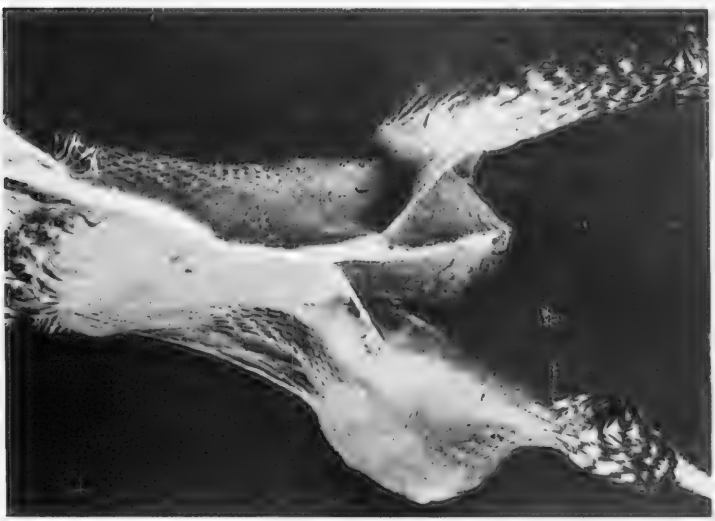


FIG. 3.

(Rous and Murphy: Causation of Chicken Tumors.)

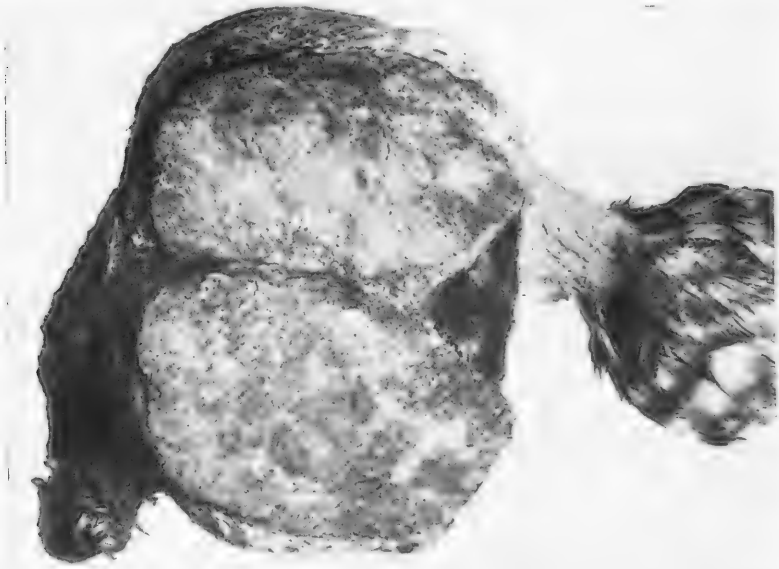


FIG. 4.

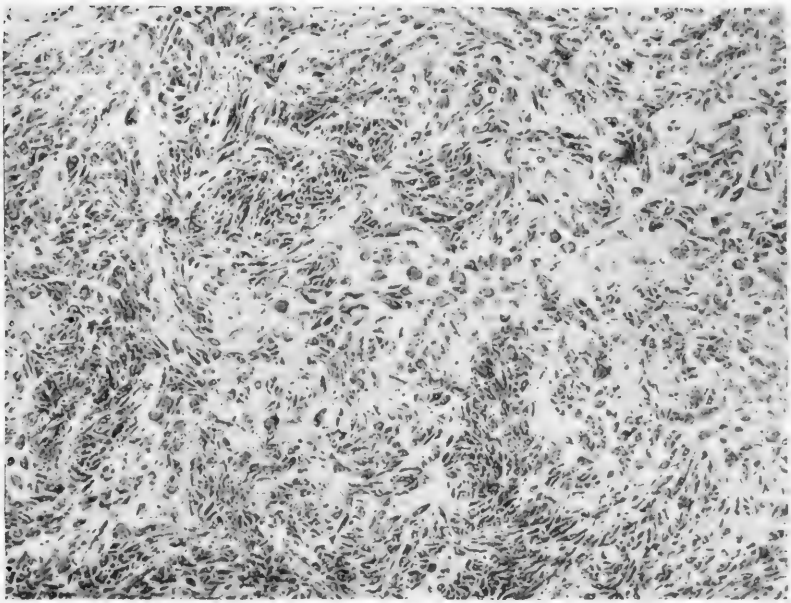


FIG. 5.

(Rous and Murphy: Causation of Chicken Tumors.

FIG. 6.

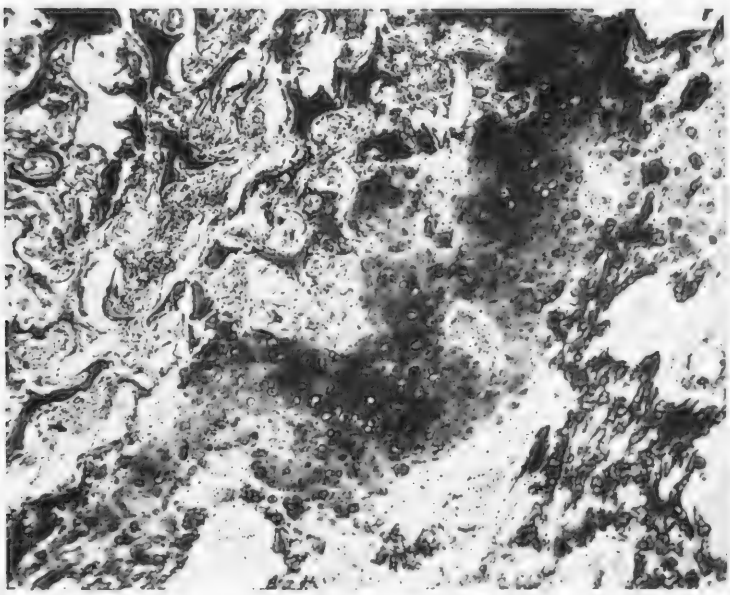


FIG. 7.

(Rous and Murphy: Causation of Chicken Tumors.)

FIG. 8.

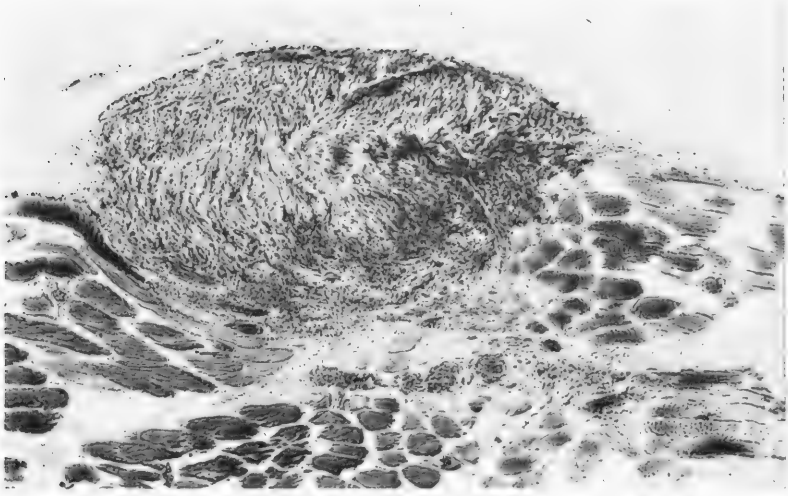


FIG. 9.

(Rous and Murphy: Causation of Chicken Tumors.)

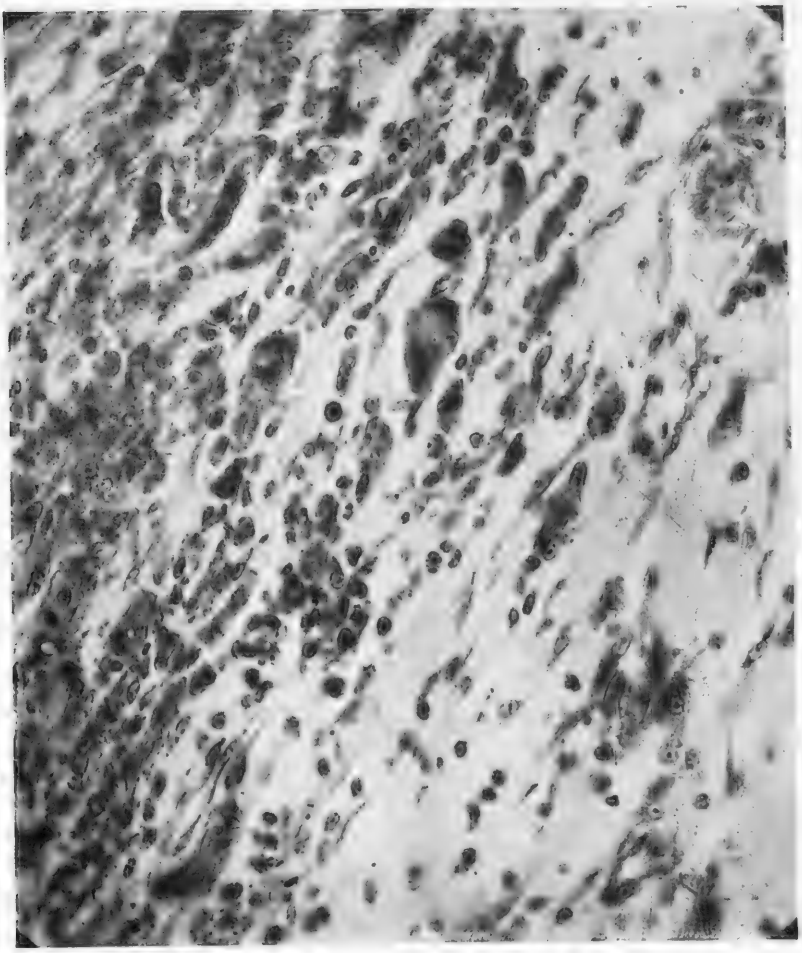


FIG. 10.

(Rous and Murphy: Causation of Chicken Tumors.)

PLATE 11.

FIG. 8. Another portion of the same growth. An unusual intermediate stage in the formation of cartilage.

FIG. 9. A spindle-celled sarcoma developing after the injection of 0.5 c.c. of a filtrate of Chicken Tumor I. No *Kieselguhr* had been added to the filtrate. The growth arose in the sheath of the outermost pectoral muscle, where the needle had been thrust through, and it was excised eleven days after the injection, at which time it measured only 0.15 cm. in diameter. There was no recurrence. The tumor is distinctly sarcomatous, sharply localized, and is enlarging to a considerable extent by expansive growth, as shown by the way in which the muscle fibers are pressed to one side. Here and there it has begun to infiltrate. About it there is a slight round-celled reaction.

PLATE 12.

FIG. 10. The border of an osteochondrosarcoma that developed after the injection of a filtrate, on the basis of a *Kieselguhr* reaction. The fowl was killed while the tumor was yet very small and before cartilage had been laid down in it. The reactive tissue has been compressed into strata by the expansive growth of the tumor. The tumor cells, which are of fibroblastic character and occupy the upper half of the picture, are invading and replacing the giant-celled, reactive tissue about the *Kieselguhr*. Numerous fragments of this latter can be seen. With the destruction of the giant cells the fragments are set free within the tumor. Here they induce no evident reaction.

# FURTHER OBSERVATIONS AND EXPERIMENTS ON GOITRE (SO CALLED THYROID CARCINOMA) IN BROOK TROUT (SALVELINUS FONTINALIS).

## III. Its Prevention and Cure.*

By DAVID MARINE, M.D.

*(From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University, Cleveland.)*

Plates 13 to 17.

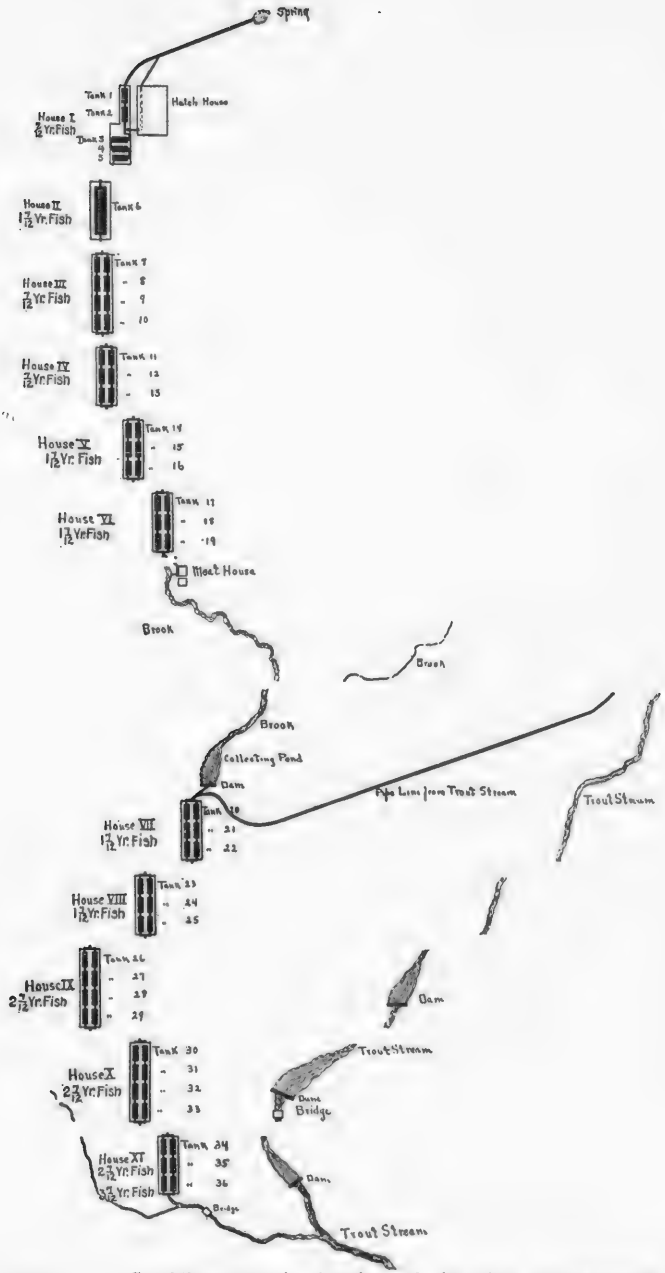
Investigations (1) made at the trout hatchery of the Blooming Grove Hunting and Fishing Club during 1909 and 1910 showed that all the fish were then goitrous. It was found that the thyroid hyperplasia began in the fry as soon as feeding was instituted, and advanced overgrowth was present at the fourth month of extra-oval life; that the overgrowth progressively increased to the stage of clinical detectability, as ascertained by the reddening of the pharyngeal floor over the thyroid area, about the tenth month in this hatchery; that visible goitres usually manifested themselves about the beginning of the second year, though they may be present as early as the sixth month, depending on the favorableness of conditions for overgrowth, and progressively increased during the second and third years; that older fish were more resistant and tended toward spontaneous recovery; that the water in which these fish lived was not naturally goitre-producing, since fish living wild in the stream and raceways did not develop thyroid overgrowth.

It was further shown that overcrowding and overfeeding with the highly abnormal and incomplete diet of hog's liver and heart were the major gross etiological factors, and of these the food was the more important factor in bringing about a fault of nutrition which stimulated the thyroid to compensatory overgrowth. No evidence was obtained that the disease was either infectious or contagious, or that a direct *contagium vivum* could account for the phenomenon.

The above summary of the work of 1909 and 1910 will serve as an introduction to the observations included in this report, which were made during August, 1913, and deal with the effect of changing the food from liver to fresh sea fish.

*Comparison of the General Conditions Prevailing in 1909, 1910, and 1913.*—The water supply has remained unchanged as regards source and volume (text-figure 1). The Club is holding more trout in each pond than in 1909 and 1910, hence the untoward factor of

* Received for publication, October 9, 1913.




TEXT-FIG. 1. Semidiagrammatic drawing of the plan, arrangements, and location of the hatchery, water supplies, houses, and retaining tanks.

overcrowding is increased. The distribution of the fish is in general the same (text-figure 1),—the fry (hatch of 1913) in the upper, the nineteen months old trout in the middle, and the thirty-one months old trout in the lower ponds. The fry have always been scrupulously cared for by daily cleaning and sweeping the troughs and ponds. With the older fish (nineteen and thirty-one months) there is clearly less detritus in the ponds than in 1909 and 1910, although little additional attention has been paid to these ponds, the difference being due mainly to the change of food. Therefore, apart from the increased number of fish being held in the same pond space and the water supply, the only important change has been in the food.

*The Food.*—Beginning with October, 1911, fresh sea fish (butter fish (*Stromateus triacanthus*) and occasionally weak fish (*Cynoscion regalis*)) have been fed six times weekly to the older fish to the exclusion of all other foods. The fry are fed two to three times daily as in previous years, with finely divided hog's liver for the first four to five months of life, then heart muscle (hog) is added to this diet. This is continued until October (*i. e.,* nine months), when all the fry are removed to the ponds. At this time the change to fish diet is also made and maintained during the remainder of their stay in captivity.[1]

[1] It is as yet impossible to change to the fish diet earlier than the ninth month on account of the inability to hash the fish finely enough with the present machinery. This will soon be remedied, however, and then much of the thyroid overgrowth that now occurs during the period of liver feeding will be prevented. Several kinds of fish have been tried as food, among which are sea bass (*Centropristes striatus*), herring (*Clupea harengus*), weak fish (*Cynoscion regalis*), and whiting (*Merlangus americanus*). All have been found unsuitable either on account of bones or scales and have been abandoned. The fish scales being unaffected by digestion form rouleaux in the gut and produce intestinal obstruction followed by the usual sequelæ of enteritis, distention peritonitis, and death. I had the opportunity of examining two mild cases of partial intestinal obstruction, although for the past year only an occasional large scaled fish has been included in the food. In the two fish examined the obstruction was intermittent, as the scales were small (from weak fish) and could only obstruct when arranged transversely in the gut. There was well developed enteritis of the straight gut with extension through to the mesentery. Bones, if fine and rigid as in herring, are not broken up by the hasher and often perforate the stomach wall before the gastric acid can decalcify them. Fish with cartilagenous endoskeletons, such as skates, rays, sharks, etc., have not been tried owing to the difficulty as yet in

*Comparison of the Gross Appearance of the Fish of 1913 with Those of 1909 and 1910.*—Very striking differences are apparent. The nineteen months and the thirty-one months old fish are active and alert, while those of corresponding ages in 1909 and 1910 were sluggish and lumbersome. In handling large numbers of them in each of these three years, the 1913 fish are clearly stronger, show more fight, fatigue less easily, and do not die so quickly when removed from the water.

The fish are now more trout-shaped, that is, they have lost the pot-bellied appearance of 1909–1910. The skin pigments are brighter and better differentiated. It will be recalled from the descriptions of 1909–1910 that in all the fish the black pigment was excessively developed. The greenish grey marbling of the back was absent and the orange and pink color spots of the sides were wholly invisible. So also the orange and yellow colors of the fins and ventral surface were nearly absent. The silvery sheen of the sides was masked by the black pigment. In the fish of 1913 the colors approach closely those of the normal trout. The greenish marbling of the dorsum is detectable. The lateral color spots are well differentiated and the yellow and orange pigment of the ventral surface and fins are developed. The silvery sheen of the sides is perhaps better developed than in the average wild trout.[2]

As regards weight I have no exact data, although the impression is distinct that they are a trifle smaller on the average than in 1909 and 1910. The fry of 1913 are to all appearances the same as on previous examinations.

obtaining them in the markets. There seems no objection to their use since they would obviate the two great objections, scales and sharp perforating bones, and in addition might be less expensive than the usual market fish. Butter fish (*Stromateus triacanthus*) have been found least objectionable of the obtainable fish and are now used almost exclusively. They are received frozen and therefore in better condition than usually obtains with liver.

[2] These colors being readily adaptable to environment are modified by the color of the water, hence native trout from the brook are slightly darker than trout living in the spring water of the hatchery. It may also be recalled that in the experiments of 1910 the excessive black pigment diminished rapidly upon changing the food and removal of the fish from the ponds to troughs or to the trout stream. It would therefore appear that the food was a factor in the excess of the black pigment developed.

Of other diseases independent of goitre, as the myxosporidian infection (Taumelkrankheit, cramps), fungus, and enlarged gills (so called sore gills), there is a definite decrease.

*Comparison of the Gross Thyroid Condition of 1909 and 1910 with That Obtaining in 1913.*—These data are obtained from the clinical examination of the thyroid areas of a series of fish from the ponds for the presence of (*a*) reddening of the pharyngeal mucosa between the first and third gill segments as the first clinical evidence of thyroid overgrowth, and for (*b*) visible goitres projecting either dorsally in the floor of the mouth or in the gills, or ventrally in the angle of the attachment of the operculum as a late sign. The fry were not thus examined.

TABLE I.

| | October 12, 1909. | | | | June 27, 1910. | | | | August 10, 1913. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pond No. | Age in yrs. | No. of fish examined. | Reddening of pharyngeal mucosa. | No. with goitre. | Age in yrs. | No. of fish examined. | Reddening of pharyngeal mucosa. | No. with goitre. | Age in yrs. | No. of fish examined. | Yellowish discoloration of pharynx. | No. with goitre. |
| 6 | | | | | 1.5 | 41 | 10 | 0 | 1.7 | 25 | 1? trace | 0 |
| 14 | | | | | 1.5 | 51 | 45 | 1 | 1.7 | 32 | 2? trace | 0 |
| 19 | 1.8 | 210 | 197 | 7 | 1.5 | 60 | 51 | 2 | 1.7 | 22 | 2 trace | 0 |
| 20 | 1.8 | 210 | 175 | 3 | 1.5 | 54 | 40 | 0 | 1.7 | 30 | 0 | 0 |
| 21 | | | | | | | | | 1.7 | 25 | 0 | 0 |
| 25 | 1.8 | 67 | 61 | 2 | 1.5 | 48 | 41 | 1 | 1.7 | 24 | 1 trace | 0 |
| 26 | | | | | | | | | 2.7 | 22 | 3 trace | 0 |
| 32 | 2.8 | 60 | 58 | 2 | 2.5 | 50 | 38 | 1 | 2.7 | 29 | 3 trace | 0 |
| 35 | | | | | 2.5 | 40 | 28 | 2 | 2.7 | 32 | 4 trace | 0 |
| 36 | 1.8 | 210 | 183 | 4 | | | | | 3.7 | 16 | 3 | 0 |
| Totals | | 757 | 89% | 0.23% | | 344 | 74% | 0.17% | | 257 | 0.07% | 0.0% |

From the above tabulation it is seen that in 1909 89 per cent. of the fish had distinct reddening of the pharyngeal floor, and 0.23 per cent. had visible goitres. In 1910 a similar examination of fish from the same ponds and of nearly the same ages showed distinct reddening of the pharyngeal floor in 74 per cent. and visible goitres in 0.17 per cent. In 1913 a third examination of the fish from the same ponds and of approximately the same ages as those of 1909 and 1910 showed only a slight yellowish discoloration of the pharyngeal floor[3] in 0.07 per cent., and visible goitres in none.

[3] The reddening of the pharyngeal floor is due to the vascular thyroid tissue growing up to the pharyngeal mucosa. The yellow discoloration of the same region is due to the thyroid follicles rich in colloid and decreased vascularity, *i. e.*, recovery or colloid stage of a preceding hyperplasia.

These percentages illustrate the average condition of all the fish at the time of the examinations and are controlled by the histological examination of specimens removed at the same time from all the ponds during each of these years. The differences in the gross condition of the thyroids for the years 1909 and 1910 are unimportant but illustrate the severity and extent of the disease. The differences in the thyroid conditions of 1909–1910 and of 1913 were beyond what I had expected and demonstrate clearly that the disease in the 1913 fish had been completely arrested. There were none with reddening of the floor of the pharynx nor with goitre, hence none with any active overgrowth of the thyroid. The few instances where slight yellowish discoloration was present is indicative of colloid-filled follicles beneath the mucosa. How can this most striking difference in the thyroid condition be accounted for? The only noteworthy determinable factor of difference between the conditions prevailing in 1909 and 1910 and those of 1913 is the food,—sea fish in 1913 and liver in 1909 and 1910. That the change of food accounts for the differences in the thyroid condition will become more apparent from the following observations and comparisons of the histological conditions of the thyroids of the entire pond series for the three years.

*Observations on the Histological Condition of the Thyroids from Specimens from All Ponds.*—(Figures 1, 2, and 3, and table II.) For comparison I have tabulated the anatomical state of the thyroids in 1909 and 1910 with that of 1913. In this table all the fish are included: (1) the fry, (2) the one to two years old, and (3) the two to three years old fish.

In the case of the fry for 1909–1913, all conditions, including food, water, crowding, cleaning, and general hygienic conditions are as nearly identical as it is possible to keep them. This is most fortunate in that it makes comparisons of the thyroid conditions for each year possible under constant conditions. It also gives a broad foundation for estimating the average thyroid hyperplasia present in the fry and is therefore valuable for checking any changes found in the older fish. With the one to two years old trout and the two to three years old trout of 1913 all conditions except the food are similar to those of 1909 and 1910. The one to two years old trout of 1913 have been fed sea fish for the past ten months. The two to three years old trout have been fed sea fish for twenty-two months, while the one to two years and two to three years old trout of 1909 and 1910 had been fed with liver only.

All the fry of 1909, 1910, and 1913 exhibited the same general thyroid condition of active hyperplasia (figures 4, 5, and 6). The thyroid area is completely filled with thyroid tissue and extension to the adjacent bone and muscle is present

in all. The differences in the different years are only of slight degree and are probably dependent on the slight age differences at the time of examination: 1909, 8 to 9 months old; 1910, 6 months old; 1913, 7 months old. The individual variations within a given year are also slight and in general the same for all years. The examinations in each year comprised two specimens taken without choice from the twenty-one troughs in the hatch house and thirteen pairs of ponds of the pond series for the fry and also two specimens for each pond of the remaining twenty-three pairs for the older fish. From the examination of the fry in 1910, which, in addition to the above, included a series taken at weekly intervals from the time of hatching (January 15) to October, it was shown that the thyroid overgrowth began as soon as the fish began feeding and was easily detectable at the fourth month of extra-oval life, whence it progressively increased through the following months of the first and second years, rarely becoming clinically detectable before the tenth month at this hatchery. In some hatcheries the overgrowth has been clinically detectable at the sixth to the seventh month, depending on more favorable conditions for thyroid overgrowth. The fry of 1911 and 1912 were not examined, but these same fish were included in the 1913 examination as thirty-one months and nineteen months old fish, respectively, and from this examination there is complete evidence that their thyroids had undergone changes identical with those in the years 1909, 1910, and 1913. Passing now to the nineteen months old fish, one finds a most striking change also noted in the gross examination of the thyroid area. All the follicles are in the colloid state, although their distribution extends into the bone and muscle and often up to the pharyngeal mucosa. The fish from the same ponds and of approximately the same age in 1909 and 1910 all had well marked active hyperplasia (figures 7 and 8) greater in amount and more widely distributed in muscle, bone, gills, and pharyngeal mucosa than those of 1913.The growth in nineteen months old fish of 1913 (compare figures 9 and 10 with figures 11 and 12) was found to be completely arrested, and the hyperplasia had completely involuted, while in the fish of 1909 and 1910 of the same age the growth was extending rapidly. As the arrest of the growth corresponds in time with the change in food, and as no other factor of difference is present, one is forced to the conclusion that the food is the major cause of the change. While the fish have grown rapidly during the ten months since the feeding of fish began, their thyroid tissue is no greater in amount nor more extensive in distribution than that reached during the first ten months of life. So also with the thirty-one months old fish which have been fed fish for twenty-two months the thyroids are completely involuted to the colloid state and the amount and distribution of the follicles are the same as those of the nineteen months old fish, and hence no greater than that attained during the first ten months of life, although the fish have made their normal annual growth. One sees, therefore, fish nineteen months and thirty-one months old with thyroids identical in all particulars and no greater in amount nor more extensive in distribution than those of liver-fed fry of ten months in this hatchery. That the growth was arrested and involution started at the time of the change from liver to sea fish cannot be doubted, although for the sake of completeness it will be necessary to make a series of weekly examinations beginning before and continuing for some months after the change of food to fresh sea fish. This is now being done. Additional proof that change

TABLE II.

| Pond or trough No. | 1909. Age of fish in months. | 1909. Condition of thyroid. Extent of overgrowth. | 1909. Condition of thyroid. Classification. | 1910. Age of fish in months. | 1910. Condition of thyroid. Extent of overgrowth. | 1910. Condition of thyroid. Classification. | 1913. Age of fish in months. | 1913. Condition of thyroid. Extent of overgrowth. | 1913. Condition of thyroid. Classification. |
|---|---|---|---|---|---|---|---|---|---|
| Trough 1 | 8 | All subpharyngeal structures,—bone and muscle | Marked hyperplasia | 6 | Entire aortic area; no invasion of bone or muscle | Moderate hyperplasia | 7 | Entire aortic area; invasion of bone and muscle | Marked hyperplasia. |
| Trough 5 | 8 | All subpharyngeal structures,—bone and muscle | Marked hyperplasia | 6 | Entire aortic area: no invasion of bone or muscle | Moderate hyperplasia | 7 | All subpharyngeal structures in bone and muscle | Marked hyperplasia. |
| Trough 10 | 8 | Entire aortic area including bone and muscle | Marked hyperplasia | 6 | Entire aortic area; invasion of bone and muscle | Marked hyperplasia | 7 | All subpharyngeal structures in bone and muscle | Marked hyperplasia. |
| Trough 15 | 8 | Entire aortic area including bone and muscle | Marked hyperplasia | 6 | Entire aortic area; invasion of bone and muscle | Marked hyperplasia | 7 | Entire aortic area | Moderate hyperplasia. |
| Trough 20 | 8 | Entire aortic area including bone and muscle | Marked hyperplasia | 6 | Entire aortic area; invasion of bone and muscle | Marked hyperplasia | 7 | Entire aortic area | Marked hyperplasia. |
| Normal above all ponds | 20 | Scattered follicles about aorta, filled with colloid | Normal | 18 | Scattered large follicles in aortic area, filled with colloid | Normal | 19 | Scattered large follicles in aortic area filled with colloid | Normal. |
| Pond 1 | 8 | Entire thyroid area packed with follicles, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled; invasion of bone and muscle | Marked hyperplasia | 7 | Entire aortic area filled; invasion of bone | Moderate hyperplasia. |
| Pond 2 | 8 | Entire thyroid area packed with follicles, in bone and muscle | Marked hyperplasia | 6 | Thyroid area filled | Moderate hyperplasia | 7 | Entire aortic area filled; invasion of bone | Moderate hyperplasia. |
| Pond 3 | 8 | Entire thyroid area filled | Moderate hyperplasia | 6 | Thyroid area filled; invasion of bone | Moderate hyperplasia | 7 | Entire aortic area filled; invasion of bone | Marked hyperplasia. |
| Pond 4 | 8 | Entire thyroid area filled | Moderate hyperplasia | 6 | Thyroid area filled; invasion of bone | Moderate hyperplasia | 7 | Entire aortic area filled; invasion of bone | Moderate hyperplasia. |
| Pond 5a | 8 | Entire thyroid area filled | Moderate hyperplasia | 6 | Thyroid area filled; invasion of bone | Moderate hyperplasia | 7 | Entire aortic area filled; invasion of bone | Moderate hyperplasia. |

TABLE II.—*Continued.*

| Pond or trough No. | 1909. | | | 1910. | | | 1913. | |
|---|---|---|---|---|---|---|---|---|
| | Age of fish in months. | Condition of thyroid. | | Age of fish in months. | Condition of thyroid. | | Age of fish in months. | Condition of thyroid. | |
| | | Extent of overgrowth. | Classification. | | Extent of overgrowth. | Classification. | | Extent of overgrowth. | Classification. |
| Pond 5b | 8 | Entire thyroid area filled | Moderate hyperplasia | 6 | Thyroid area filled; invasion of bone | Moderate hyperplasia | 7 | Entire aortic area filled; invasion of bone | Moderate hyperplasia. |
| Pond 6 | 8 | Entire thyroid area filled | Moderate hyperplasia | 18 | Entire thyroid area filled, in bone and muscle; infoldings; no colloid | Marked hyperplasia | 19 | Numerous small and large colloid-filled follicles in entire thyroid area | Colloid goitre. |
| Pond 7 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire thyroid area filled, in bone | Moderate hyperplasia | 7 | Aortic area filled; extension to bone | Moderate hyperplasia. |
| Pond 8 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled, in bone and muscle | Marked hyperplasia | 7 | Aortic area filled; extension to submucosa | Marked hyperplasia. |
| Pond 9 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled, in bone and muscle | Marked hyperplasia | 7 | Aortic area filled; extension to submucosa | Marked hyperplasia. |
| Pond 10 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled, in bone and muscle | Marked hyperplasia | 7 | Aortic area filled; extension to submucosa | Marked hyperplasia. |
| Pond 11 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled, in bone and muscle | Marked hyperplasia | 7 | Aortic area filled; extension to submucosa | Marked hyperplasia. |
| Pond 12 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled, in bone and muscle | Marked hyperplasia | 7 | Aortic area filled; extension to submucosa | Marked hyperplasia. |
| Pond 13 | 8 | Entire thyroid area filled, in bone and muscle | Marked hyperplasia | 6 | Entire aortic area filled, in bone and muscle | Marked hyperplasia | 7 | Aortic area filled; extension to submucosa | Marked hyperplasia. |
| Pond 14 | 20 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 18 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 19 | Numerous colloid-filled follicles in entire aortic area | Colloid goitre. |
| Pond 19 | 20 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 18 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 19 | Numerous colloid-filled follicles in entire aortic area | Colloid goitre. |

| Pond or trough No. | 1909. | | | 1910. | | | 1913. | |
|---|---|---|---|---|---|---|---|---|
| | Age of fish in months. | Condition of thyroid. | | Age of fish in months. | Condition of thyroid. | | Age of fish in months. | Condition of thyroid. | |
| | | Extent of overgrowth. | Classification. | | Extent of overgrowth. | Classification. | | Extent of overgrowth. | Classification. |
| Pond 20 | 20 | Entire subpharyngeal space filled, in bone and muscle | Colloid, moderate hyperplasia (hyperplasia involuting) | 18 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 19 | Numerous colloid-filled follicles in entire aortic area | Colloid goitre. |
| Pond 21 | 20 | Entire subpharyngeal space filled, in bone and muscle | Colloid, moderate hyperplasia (hyperplasia involuting) | 18 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 19 | Numerous colloid-filled follicles in entire aortic area | Colloid goitre. |
| Pond 25 | 20 | Entire subpharyngeal space filled, in bone and muscle | Colloid, moderate hyperplasia (hyperplasia involuting) | 18 | Entire subpharyngeal space filled, in bone and muscle; visible goitre | Marked hyperplasia | 19 | Numerous colloid-filled follicles in entire aortic area | Colloid goitre. |
| Pond 26 | 32 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia, small visible goitre | 30 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 31 | Numerous colloid-filled follicles in entire aortic area; number of follicles same as in 19 mos. old fish | Colloid goitre. |
| Pond 32 | 32 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 30 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 31 | Numerous colloid-filled follicles in entire aortic area; number of follicles same as in 19 mos. old fish | Colloid goitre. |
| Pond 35 | 32 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia, slight general fibrosis | 30 | Entire subpharyngeal space filled, in bone and muscle; visible goitre | Marked hyperplasia | 31 | Numerous colloid-filled follicles in entire aortic area; number of follicles same as in 19 mos. old fish | Colloid goitre. |
| Pond 36 | 20 | Entire subpharyngeal space filled, in bone and muscle | Marked hyperplasia | 30 | Entire thyroid area filled, extension to skin, bone and muscle | Marked hyperplasia | 43 | Entire subpharyngeal area filled with colloid follicles, in bone and muscle; number of follicles much greater than in the 19 or 31 mos. old fish | Colloid goitre. |

of food is the cause of the arrest of thyroid growth is the preservation of 250 forty-three months old fish which had been fed liver for the first twenty-one months of life and sea fish for the last twenty-two months. In these I found three out of the sixteen examined having distinct yellowish discoloration of the pharyngeal floor, and in the two specimens examined histologically the thyroid was completely involuted to the colloid state; but the amount and distribution were far more extensive than in the thirty-one and nineteen months old fish, showing that the thyroid overgrowth had progressed much farther before the involution began, and corresponds to the extra year of liver as food.

It is well known that foods influence the mammalian thyroid to a marked degree. Baumann (2) noticed that flesh diets stimulate the thyroid in dogs to active hyperplasia. I have repeatedly made such observations, liver being the most important food in this regard. Reid Hunt (3) in his acetonitrile experiments noted that liver stimulates the thyroid of white mice. Watson (4) has described the effect of different diets on rat thyroids and also noted that meats produce hyperplasia, while mixed diets do not. I have made similar observations. Dogs spontaneously recover from goitre on a mixed diet, while meats, especially liver, maintain hyperplasias. In zoölogical gardens where carnivores are held and bred in captivity and the diet is for the most part beef, goitre, rickets, and osteomalacic states are quite common. Foods modify the thyroid slowly, involution extending over months, while iodin involutes hyperplasias much more rapidly. Iodin administered to dogs, sheep, pigs, and human beings often induces involution in a month, and in Lake Erie pike the involution of mild degrees of hyperplasia takes place in twenty-six days (5). In brook trout with extensive thyroid overgrowth and visible goitres involution occurs in forty days.

To summarize, it is seen that the ages of the fish held at the hatchery in August, 1913, are seven months, nineteen months, thirty-one months, and forty-three months. The seven months old fry all have active hyperplasia not different in degree or extent from the fry of 1909 and 1910. The nineteen, thirty-one, and forty-three months old fish all have completely involuted or colloid thyroids, and the amount and distribution of the thyroid tissue in the nineteen and thirty-one months old fish are the same and represent the degree of thyroid overgrowth reached during the first nine months of life when liver and heart only are fed. The forty-three months old fish had been fed with liver and heart for the first twenty-one months of life, and the thyroids both on gross and microscopical examinations were much larger and more widely distributed than in the nineteen and twenty-one months old fish.

## DISCUSSION.

These data afford supplementary proof for several of the conclusions deduced from the earlier work, and in addition establish another simple and certain means for the cure and prevention of goitre in fish.

The view previously held by some observers that this disease was closely related to true carcinoma is now only of theoretical interest,

since, in addition to the facts already reported, it establishes the fact that the feeding of fresh sea fish also readily arrests the disease. No tissue overgrowth that presents the biological phenomena described for this disease, which are also identical with those of mammalian goitre, can be considered malignant. It was urged by some observers that the reaction with iodin could not be utilized as proof that the disease was not cancer since arsenic, mercuric chloride, and colloidal copper were reported to have the same thyroid effects (6). I have not been able to affect these changes with arsenic in canine goitre. I (7) have also failed to modify the growth of the mammalian thyroid carcinoma by the use of iodin, and from an extensive experience with human thyroid adenomata the conclusion was reached that they also are rarely affected by iodin (8). On the other hand, simple physiological hyperplasias of all animals are readily and invariably modified by iodin.

This disease should be classified as endemic goitre or endemic thyroid hyperplasia. One must recognize all degrees of the overgrowth in fish, just as in mammals, from the slightest departure from normal, detectable only microscopically, to the most extensive infiltration of the thyroid region and the formation of external goitres. Whether this form of goitre is similar to ordinary goitre in birds and mammals is not known. As mentioned above, they are identical anatomically, physiologically, and pathologically in all their known reactions, but inasmuch as goitre is only the symptomatic manifestation of a nutritional disturbance it is possible that many agents are capable of exciting the thyroid to this single and only known anatomical manifestation of increased activity, compensatory hypertrophy and hyperplasia.

From our knowledge of mammalian goitre it would seem probable that the thyroid reaction in infectious diseases, puberty, pregnancy, cretinism, Basedow's syndrome, etc., are not due to the same exciting cause. The fundamental fault in nutrition is probably the same in all, but the immediate exciting agent is probably different. In mammalian goitre the localization in certain districts is most obvious, while in fish and in birds this is not so apparent although it is present. Goitre, however, occurs wherever favorable conditions are created whether in mammals or in the lower animals. It is

therefore the increased susceptibility of mammals to goitre that brings out the localization of endemicity. Fish are very resistant to goitre and only acquire the disease when subjected to conditions incompatible with prolonged life in mammals.

The nearest approach to the etiology of goitre in fish was made when it was shown that food is the major etiological factor. Fish fed exclusively with liver always acquire thyroid hyperplasia, while fish of the same age, breed, and environment readily recover or escape goitre when fed with whole sea fish.

It is almost certainly a biochemical reaction, but whether it is due to the presence or absence of some substance normally needed by the organism is not known. A comparison of liver as a food with fish as a food seems to indicate that the liver lacked something normally needed by the developing fish which the diet of fish contained.

It is also probable that the liver contains some substances in excess, in attempting to utilize which, the animal exhausts other elements necessary for nutrition which are not present in the liver in sufficient amounts. As to the nature of this chemical fault one cannot reasonably speculate. From time to time most of the inorganic and many of the organic substances have been specified as etiological factors, but without foundation. Some observers have called it a toxin, but work based upon this hypothesis has added nothing to our knowledge of the etiology.

Iodin is certainly reduced in fish with thyroid hyperplasia, as in all other animals, and iodin certainly prevents and involutes hyperplasia, but the conclusion that goitre is due to a deficiency of iodin is not justified since there is considerable evidence to show that some other factor or factors are operating to divert or deplete an otherwise sufficient amount of iodin. On the other hand, the beneficial effects of feeding fish may be due to the iodothyreoglobulin that it contains, since only exceedingly small amounts are necessary, and the whole fish including the thyroid gland is used. Sea fish may also contain traces of iodin apart from the thyroid. Therefore much careful work must still be done to exclude iodin as the cause of the beneficial effects. I have made two iodin determinations on

mixed specimens of whole butter fish, and two determinations on mixed specimens of fish from which the thyroid areas had been removed. The results are as follows: (1) whole fish, slightest trace; (2) exclusive of thyroid area, no trace of iodin.

The possibility of a *contagium vivum* in fish goitre can, it seems to me, be eliminated since there is not the slightest evidence that it is either contagious or infectious. It obeys none of the laws which we associate with true infectious processes. No evidence of natural or acquired immunity has been obtained, though different species of fish exhibit different degrees of resistance (carp, trout).

Gaylord (9) has reported the finding of evidences of immunity, but this evidence does not exclude the following important factors: (1) Age is an important factor in all animals; the young are more susceptible to goitre and the old tend toward spontaneous recovery. (2) When the active hyperplasia involutes to the colloid state it is more difficult to produce again active hyperplasia (*a*) because of the increased age of the animal, and (*b*) because of the increased factor of safety due to the increased number of thyroid follicles over what obtains normally. For example, if a dog has fifty grams of colloid thyroid it is more difficult, apart from any age factor, to induce active hyperplasia in it than if the dog has three grams of normal thyroid; but other things being constant, three grams of colloid will undergo hyperplasia as readily as three grams of normal thyroid. (3) The food is probably the most important factor in determining the onset or cessation, progression or regression of the hyperplasia. (4) Water supply has some influence on the resistance of fish to active hyperplasia, reduced and much used water favoring, and large amounts lessening it. (5) The different Salmonidæ vary greatly in their susceptibility. I have seen none that were not susceptible, but the brown trout and rainbow trout in my experience have been more resistant than the silver salmon or brook trout.

The water supplied to this hatchery has little if anything to do with the development of goitre, since the fish have never developed the disease unless the single factor of overfeeding with the highly abnormal diet of liver and heart is also operating. The following observations bearing on the relation of water to the etiology and spread of goitre may be mentioned: (1) Within the hatch house are

twenty-one troughs,—twelve hatch troughs and nine nursing troughs. The twelve hatch troughs receive water directly from the spring, while the nine nursing troughs receive the water from the twelve hatch troughs. Every summer these troughs are used to hold extra fry. They are kept remarkably clean, yet all the fry have shown equally marked thyroid overgrowth in each of the years that they have been examined. Fish living outside the troughs and ponds (hence not directly fed with liver), whether above, below, or between the troughs and ponds, never develop active hyperplasia, although in most instances they have some degree of colloid goitre, showing that they had once been confined in the troughs or ponds and that, after escaping, the hyperplasia involutes. (2) The 1913 distribution of the fish made another observation possible. In house II (text-figure 1) there are 3,000 nineteen months old fish which have been fed with fish for the past ten months; all these fish have colloid glands. While above in house I and below in house III there are seven months old fry which have been fed only with liver and heart muscle and all have well marked active thyroid hyperplasia. There is also some evidence that liver when fed in excessive amounts is a more potent stimulus than when fed in small amounts, as the fish living in the runways between the houses also obtain small particles of liver which float through the screens but do not develop active hyperplasia. Plehn (9) has reported greater variations in the occurrence and distribution of the disease in a fish hatchery than they could account for purely on the basis of external changes. They suggest that these variations point to infection. I have also seen wide variations in its occurrence in different hatcheries and even in the same hatchery, but feel that all the variations could be accounted for on the basis of one or more of the following factors: (1) food, (2) water supply, (3) age, and (4) species of fish. Some observers have applied the term epidemic goitre to this form of goitre in fish. This is wrong. There is nothing acute or localized in its occurrence or distribution. It appears wherever fish are overfed with liver or other incomplete foods. It extends over the entire time of such feeding and all fish are affected.

Goitre has been present in this hatchery for the past twenty-five

years and, in my opinion, it will remain as long as fish of any age are fed with liver.

*Treatment and Prevention.*—Trout living in their normal environment and partaking of their normal food do not develop goitre, hence in the treatment and prevention our efforts should be directed toward imitating natural conditions as far as possible. However, as with many other animals it has been found possible to alter these natural environmental conditions within extremely wide limits without seriously impairing their health. Goitre is one of the serious diseases that has developed in testing the range of adaptability. The infectious diseases of fish, as of mammals, are better known and methods for the cure and prevention have been studied longer. Goitre belongs to the group of diseases dependent upon nutritional disturbances, and our knowledge of the food requirements of animals generally and of fish in particular is as yet in its infancy.

It has been conclusively shown that the feeding of the highly artificial and incomplete diet of liver and heart muscle is the major factor in the causation of fish goitre, and the first essential in treatment is to provide some other food that meets the animal's requirements. A natural food of trout is fish and the experiments of the past two years show that when sea fish is fed to these trout existing goitre is cured and the development of goitre is wholly prevented.

The feeding of fish with hard fine bones or with large heavy scales is associated with risks mentioned above. The fish stomach is adapted for the ingestion of relatively large food boli, and food should be fed in as large masses as they will take since digestion is a continuous process in fish. This would probably solve the bone question and would tend to control overfeeding. Some sort of chopper should be devised instead of hashers, since hashers mince the food and allow of too rapid digestion and, therefore, too long intervals of stomach inactivity. Coarse fish, like sharks, rays, etc., might be utilized. Sea fish, therefore, makes a perfect food as far as the cure and prevention of goitre are concerned. Overfeeding must be guarded against. Definite amounts of food should be fed but once a day with a fast day each week comparable to the fast day maintained among the carnivores at Zoölogical Gardens. No obser-

vations have been made as to the minimum amounts of sea fish necessary to involute or prevent hyperplasia, but judging from the rapidity of the involution when the food is changed to fish only, it would appear that the daily feeding with fish was not necessary to cure or prevent the hyperplasia. It might be beneficial to alternate between fish and liver or between fish and other foods daily or weekly. Overcrowding is dependent on the water supply and the food. It is a highly important factor as regards the general health of the fish, the prevention of traumata and infectious diseases, the oxygen supply, etc., but is only a minor factor in the production of goitre.

Cleanliness likewise is a secondary factor in goitre, but is of great importance in the prevention of other diseases and as a general hygienic measure cannot be neglected. Modification of pond construction with this in view would greatly simplify the cleaning and sanitary problems. Iodin in minute traces has also been found to prevent and cure goitre. Indeed it is a specific therapy for goitre but does not relieve other untoward manifestations of an incomplete food. Gaylord (6) has reported that arsenic and mercuric chloride also effect similar changes in fish thyroid hyperplasia. Our observations with arsenic in canine goitre were negative and in any event the action of arsenic and mercuric chloride is not comparable to the action of iodin on the thyroid.

## CONCLUSIONS.

1. Goitre in fish is a non-infectious, non-contagious, symptomatic manifestation of a fault of nutrition, the exact biochemical nature of which has not been determined.

2. Feeding the highly artificial and incomplete diet of liver is the major etiological factor in bringing about this fault of nutrition which is at once corrected by feeding whole sea fish.

3. Water plays no essential part in the etiology, transmission, or distribution of the disease in the fish of this hatchery.

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## EXPLANATION OF PLATES.

### Plate 13.

Fig. 1. Rough projection sketch of a transverse section through the thyroid area of a brook trout with a normal thyroid.

Fig. 2. Rough projection sketch of a transverse section through the thyroid area of a brook trout with a mild degree of active hyperplasia.

Fig. 3. Rough projection sketch of a transverse section through the thyroid area of a brook trout with a mild degree of colloid (goitre) gland.

### Plate 14.

Fig. 4. Transverse section of the thyroid area of a five months old brook trout showing the extent of the thyroid overgrowth at this age in fry of the hatch of 1910.

Fig. 5. Higher magnification of figure 4, showing the filling up of the thyroid area, the extension to the cuticle, to the pharyngeal mucosa, and on the right the extension to the gill filaments. Note the relation of the follicles to the two terminal branches of the ventral aorta, the size of the follicles, the high columnar epithelium with infoldings and plications, and the absence of stainable colloid.

PLATE 15.

FIG. 6. Transverse section of the thyroid area of a seven months old brook trout of the hatch of 1913 showing the pharyngeal mucosa above and gill filaments laterally. Note the distribution of the hyperplastic follicles about the ventral aorta and their irregular distribution throughout the thyroid area.

FIG. 7. Transverse section of the thyroid area of a seventeen months old brook trout, showing the whole thyroid area uniformly filled with hyperplastic thyroid follicles.

PLATE 16.

FIG. 8. Higher magnification of an area from figure 7, showing the infoldings and plications, vascularity, high columnar epithelium, absence of stainable colloid, and the great distortions and variations in the size of rapidly growing thyroid follicles.

FIG. 9. Transverse section of the thyroid area of a nineteen months old brook trout which during the first nine months of life had active hyperplasia, and upon the introduction of fish as the food all the follicles involuted back to the colloid state. Note the distribution and numbers of follicles as those attained during the stage of active hyperplasia, while the individual follicles now resemble in all essentials normal follicles. Note also the extension into bone and muscle. This is the colloid, or resting, or cured stage of a mild degree of active hyperplasia.

PLATE 17.

FIG. 10. Higher magnification of a portion of figure 9, showing part of the ventral aorta and the large venous space to the left. Compare the numbers and distribution of the follicles, their colloid contents, and their epithelial investments with those of figure 12.

FIG. 11. Transverse section through the thyroid area of a nineteen months old brook trout, showing the pharyngeal mucosa above, gill filaments laterally, and scattered thyroid follicles of different sizes widely distributed about the ventral aorta. Normal thyroid.

FIG. 12. Higher magnification of an area from figure 11, showing the size and arrangements of the follicles together with the colloid contents and epithelial investments.

FIG. 3. (Marine: Goitre in Brook Trout.)



FIG. 2.



FIG. 1.

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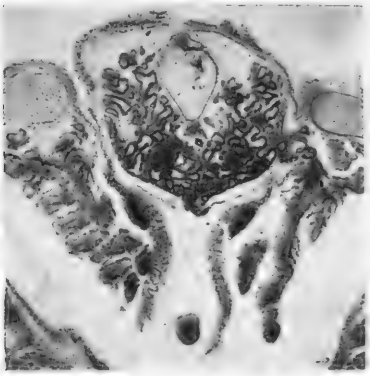

FIG. 4.

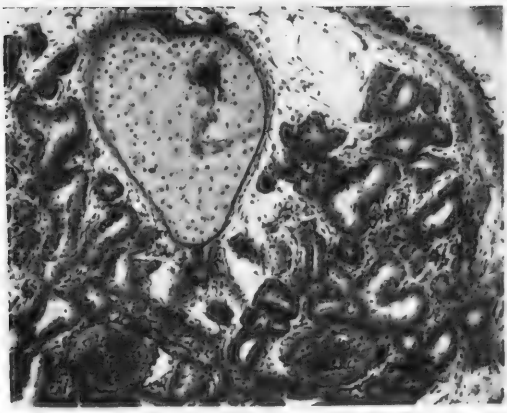

FIG. 5.

(Marine: Goitre in Brook Trout.)

FIG. 6.

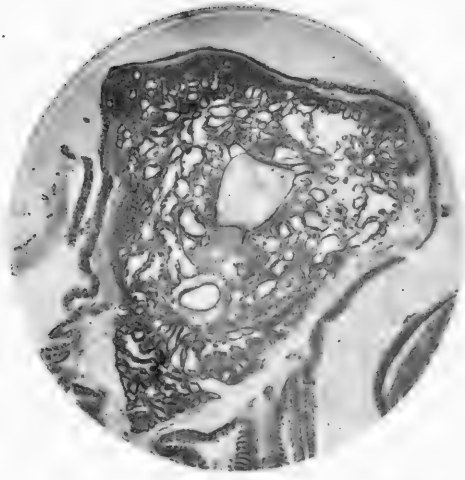

FIG. 7.

(Marine: Goitre in Brook Trout.)

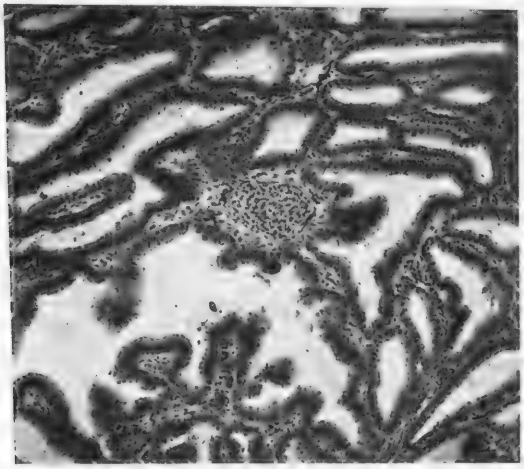

FIG. 8.

FIG. 9.

(Marine: Goitre in Brook Trout.

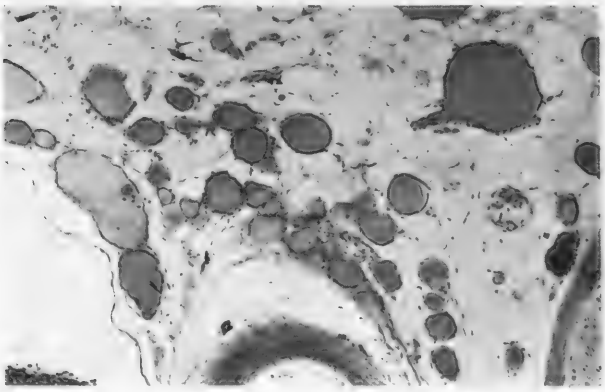

FIG. 10.

FIG. 11.

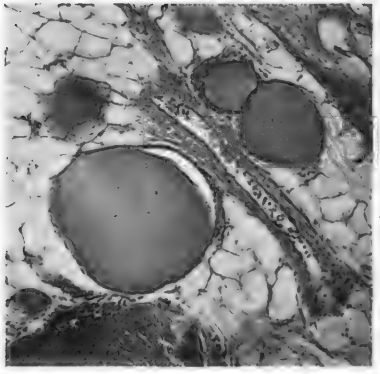

FIG. 12.

(Marine: Goitre in Brook Trout.)

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# OBSERVATIONS ON TETANY IN DOGS.

## Relation of the Parathyroids to the Thyroid; Relation of Tetany to Age, Amount of Parathyroid Tissue Removed, Accessory Parathyroids, Pregnancy, Lactation, Rickets, Sulphur, and Diet; Relation of Parathyroids to Sugar Tolerance; Effect of Calcium Salts.*

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### THE DISTRIBUTION OF PARATHYROID TISSUE AND THE RELATION OF THE PARATHYROIDS TO THE THYROID.

As a complicating factor in thyroid work during the past eight years I have observed thirty-eight cases of tetany and three instances where total removal of the thyroid lobes was not followed by any of the phenomena of tetany.

The frequency of tetany following thyroidectomy in dogs is an index to the constancy of location of the parathyroid glands. On account of the fact that in most of these cases partial thyroparathyroidectomy was done from time to time extending over months and sometimes years before the insufficiency became clinically detectable, ample opportunity was offered for compensatory adjustment, and in this way some cases escaped that would otherwise have had at least a transient tetany if the same amount of parathyroid tissue had been removed suddenly.

In all carnivora it is exceedingly difficult to remove totally even the normal thyroid without obtaining at least a transient tetany on account of the intimate anatomical relation of the parathyroids to the thyroid, while in man and the herbivora this difficulty rarely arises on account of the wide separation of the inferior or external or IIId parathyroids from the thyroid lobes. In dogs the difficulty is of course greatly increased when the thyroid lobes are enlarged, as they are in about 90 per cent. in this locality. There is much writ-

* Received for publication, October 9, 1913.

ten concerning the distribution of the parathyroid tissue. Its origin from four separate anlagen enhances the possibilities for abnormal distribution outside the thyroid area. In dogs aberrant parathyroids are most frequently found in connection with the thymus. In one series of routine examinations of ninety-eight thymuses, I accidentally included parathyroids in the specimens for histological examinations on two occasions and in a case of transient tetany following removal of both lobes of the thyroid I found at post-mortem examination 257 days later a large accessory parathyroid in the thymus. In the three instances (A–106, A–123, and A–194) where following complete removal of both lobes not even transient tetany developed, no parathyroid tissue was found at the routine post-mortem examination, though several suspected bits of tissue were examined histologically.

Parathyroid tissue unconnected with the thyroid lobes in sufficient amounts to maintain life after the removal of both thyroid lobes is present in at least 5 to 6 per cent. of dogs. This percentage is further increased (1) by partial parathyroidectomy extending over months and years which allows of the development of any compensatory mechanism, and (2) by the temporary use of calcium salts which will occasionally tide the animal over an otherwise fatal tetany.

These facts concerning the distribution of parathyroid tissue, and the influence of compensatory hypertrophy and calcium salts are of importance in the controversy that has arisen as to the relation of the parathyroids to the thyroid and their importance to life.

Bearing on the question of the interrelationship of the parathyroids and thyroid which some English observers, notably Vincent (1) and Forsyth (2), have supported are the only two cases of operative myxedema in dogs we have observed (both transient), and in both of these transient and partial tetany not associated with the myxedema developed. The cases are given below.

*Dog A–112.*—Female fox terrier. Jan. 11, 1908. Weight 5.5 kilos. Jan. 15. Entire left lobe and two thirds of right lobe (weight 4.15 gm.) removed. Mar. 15. Two pups born. Mar. 25. Removed about one half of remaining portion of right lobe. Mar. 26. Violent tetany. Calcium chloride and milk given daily until Apr. 6. Oct. 29. Five pups born. Nov. 3. Violent tetany. Milk and

calcium chloride given for three days; tetany cleared up. Dec. 3. Pups weaned. Dec. 7–18. The remainder of the right lobe (weight 0.25 gm.) was removed, and 10 c.c. of saturated aqueous solution of calcium chloride were given daily. Dec. 24. Hair falling out, skin dry and itchy. Weight 5.9 kilos. At first it was thought to be mange and the dog was given sulphur rubs, but during January, 1909, alopecia became very marked and the dry skin was everywhere visible. Jan. 29. Weight 7.2 kilos. Feb. 12. Weight 7 kilos. New coat of hair is beginning to come in. Feb. 26. Weight 6.2 kilos. Mar. 15. Weight 6.1 kilos. New coat of hair about 0.5 cm. long. In heat. May 24. Two pups born. June 5. Weight 5.7 kilos. July 10. Weight 6 kilos. Aug. 10. Weight 6.2 kilos. Sept. 14. Weight 6.2 kilos. Oct. 4. Weight 5.9 kilos. Nov. 26. Weight 5.9 kilos. Dec. 9. Killed in fight with another dog.

*Autopsy.*—One large accessory parathyroid was found in the thymus, and three small aortic thyroids were in a state of marked hyperplasia.

*Dog A–183.*—Female mongrel. Nov. 16, 1909. Weight 10 kilos. Nov. 16–23. 1 c.c. of Lugol's solution given. Dec. 1. Bit of left lobe removed. Weight 0.45 gm. Dec. 1, 1909, to Feb. 9, 1910. Dog received 1 gm. of sodium cyanate daily. Dec. 15. Another bit of left lobe was removed. Weight 1.25 gm. Jan. 6, 1910. Remainder of left lobe removed (weight 2.49 gm.). Jan. 19. Bit of right lobe removed (weight 0.67 gm.). Feb. 9. Bit of right lobe removed (weight 1.58 gm.). June 13. Weight 10.2 kilos. Oct. 11. Nine pups born. Nov. 15. Pups weaned. All have strikingly enlarged thyroids. Oct. 15. Weight 12 kilos. Hair falling out. Jan. 6, 1911. Loss of hair is marked. Weight 12.2 kilos. Feb. 12. Weight 11 kilos. New hair coming in. Mar. 3. Weight 10.2 kilos. Apr. 1. Weight 9 kilos. Active; coat glossy. Apr. 11. Remaining portion of right lobe isolated, about size of a medium olive, much hypertrophied. Removed portion (weight 4.6 gm.) leaving a small stump. Apr. 13. Violent tetany. Calcium chloride solution by stomach. Apr. 18. Weight 9.6 gm. Occasional tetany sometimes mild and often severe, always controlled by calcium lactate. May 1. Impregnated. May 20. Pregnant. Suddenly taken with violent tetany; relieved by calcium lactate. Occasional twitchings until June 30, nearing full term, severe tetany, calcium lactate. July 1. Tetany again, calcium lactate. Was in tetany daily until July 6, when she died of tetany shortly after the birth of seven living full term pups.

Histological examination of the remaining stump of the thyroid lobe revealed a trace of parathyroid tissue in the dense scar tissue of the thyroid capsule. The thyroid tissue is in a state of marked hyperplasia. Two aortic thyroids are present, but no other parathyroid tissue is found.

In these two experiments the tetany was dissociated from the myxedema. In dog A–112 the last attack of tetany preceded by about forty days the onset of myxedema which occurred following the last operation for total removal of the thyroid lobes. The myxedema cleared up in three months and the dog remained in apparent health clinically until killed on December 9, thirteen months after the last attack of tetany and nine months after the disappearance of myxedema. In dog A–183 the myxedema developed after the fifth

operation of partial removal of thyroid (about nine tenths), and completely cleared up two months before the onset of tetany on the day after the sixth operation for removal of 4.6 grams of the much hypertrophied stump of the right lobe. The dog remained subtetanic with the aid of calcium salts until the end of pregnancy, when an uncontrollable parathyroid insufficiency developed and ended fatally.

Such observations when compared with the experiences of others, —Simpson (3) with tetanies in sheep; Halsted (4), MacCallum (5), Erdheim (6), and others with tetanies in dogs; Kocher (7), Reverdin (8), and Fuhr (9) with total thyroidectomy in man; Maresch (10), Erdheim (11), and Getzowa (12) with congenital defects; and Roussy and Clunet (13) with spontaneous myxedema, —leave no doubt that as regards myxedema and tetany the parathyroids and thyroids are anatomically, physiologically, and pathologically independent structures.

## THE RAPIDITY OF THE DEVELOPMENT OF TETANY AND THE RELATION OF TETANY TO AGE, AMOUNT OF PARATHYROID TISSUE REMOVED, ACCESSORY PARATHYROIDS, PREGNANCY, LACTATION, RICKETS, SULPHUR, AND DIET.

I have seen violent tetany develop as early as seven hours after the removal of the parathyroids (dogs A–149 and A–167) and end fatally within eleven hours of the operation. In our series the average time between the last operation and the onset of tetany was eighteen to twenty-four hours. This has impressed me as relatively more rapid than the average time intervals reported by other observers. The conditions are not quite comparable as usually the parathyroidectomy is made at a single operation, while in nearly all of our cases partial thyroparathyroidectomy had been practiced for weeks and months and occasionally a year before the final operation precipitating the tetany. It is therefore possible that the factor of safety or protective reserve was greatly reduced before the final operation.

Several factors influence the rapidity of onset of tetany: (1) age, (2) the amount of parathyroid tissue removed, (3) the presence of inaccessible accessory parathyroid tissue, (4) pregnancy and lactation, (5) rickets, (6) the use of sulphur, and (7) the diet.

*Age.*—The age of the animal, as many observers have pointed out, is important. Simpson (3) in his work with sheep showed that lambs usually develop tetany following parathyroidectomy, while in yearlings he never observed a fatal tetany, and in old sheep there were no tetanic manifestations at all. In dogs and cats tetany follows the parathyroid removal at any age so that the age factor cannot be shown in this way. The young are, however, more susceptible. The difference due to age is suggested by the following observations. I ligated the superior thyroid arteries on both sides in four pups (A–203, A–204, A–205, and A–206) of the same age (sixty-two days) and same litter, and in all violent tetany developed on the following day, eighteen hours after the ligations. They died of tetany on the eighth, twelfth, ninth, and eighth days, respectively, although calcium lactate was given daily. At autopsy the wounds were healed *per primam.* Only the regions of the superior poles had been traumatized by the operative procedure. The thyroid lobes were somewhat enlarged in each case and in a state of marked hyperplasia. Anemic necrosis roughly involving the upper half of the lobes was present in each case. The inferior thyroid artery, which is normally very small in the dog, was intact and uninjured in all. In twelve similar operations in adult dogs undertaken to study the effect of superior thyroid artery ligation none developed tetany although there was in all a small zone of necrosis at the upper poles involving in some instances a third of the lobe. While the difference in outcome between young pups and adults may be explained principally on the difference in the extent of the necrosis still this can scarcely be the whole explanation. These pups were slightly rachitic and this also is probably an important factor.

*Amount of Parathyroid Tissue Removed.*—In adult dogs under normal conditions of food and environment one uninjured IIId parathyroid is sufficient to prevent fatal tetany. Many cases are reported where a IVth, or even smaller bit of parathyroid tissue has prevented all signs of tetany. I have seen three cases (dogs A–106, A–123, and A–194) where total removal of the thyroid lobes with their normal quota of parathyroids induced no symptoms. The routine autopsies of these cases failed to reveal any accessory para-

thyroid tissue although several suspected bits were examined histologically. The examinations were in no sense complete, as such a procedure would involve serial sections of the entire neck and upper thorax, and I am convinced that accessory parathyroids were present somewhere in the parathyroid area. On the other hand, I have seen typical and violent tetany occur in a lactating bitch, where four parathyroids were intact, as ascertained at autopsy.

It is clear from these extremes and from the larger number of cases with intermediate amounts of parathyroid tissue showing variations in the occurrence of tetany that other factors than quantity of parathyroid tissue determine the onset of tetany. Thus when an anatomical insufficiency exists there exists also a physiological insufficiency, but the reverse is often not true since a physiological or relative insufficiency may exist when two and even four parathyroids are intact.

The same is, of course, true of the thyroid tissue,—that where an anatomical deficiency exists a physiological insufficiency exists also, but in the spontaneous thyroid insufficiencies and even in the induced insufficiencies there is usually an excess of thyroid tissue in the early stages.

*Pregnancy and Lactation.*—These factors tend to induce a relative parathyroid insufficiency. In 1888 Halsted (4) observed this in his cases of partial parathyroidectomy in dogs which developed tetany late in pregnancy. Since then many workers have observed this tendency. There were five cases in this series (dogs A–112, A–113, A–132, A–161, and A–183) where tetany developed with pregnancy or lactation.

In dog A–112 partial thyroparathyroidectomy, followed by a transient tetany for four days, was done six months before the next pregnancy. The dog developed tetany early in lactation which was controlled by calcium salts. The remainder of both lobes was removed later without causing tetany, calcium salts being given for eleven days. Six months later the animal gave birth to another litter of pups without any tetany resulting.

In dog A–183 a mild tetany continued throughout the pregnancy, which was controlled until the last week by the use of calcium salts.

Then in spite of the daily use of calcium lactate the tetany became severe and terminated fatally at the birth of the full term pups. Another bitch (A–113) aborted during a severe tetany that probably could have been prevented by calcium salts.

In another case (A–132) following a first operation for the removal of one and three fourths thyroid lobes on December 7, 1908, a transient tetany developed which was controlled by the administration of calcium salts for ten days. The dog remained free from tetany during the next pregnancy and lactation (May 1 to August 1, 1909) but developed tetany on April 1, 1910, thirteen days before the birth of a second litter of pups. This was wholly controlled by calcium lactate until the birth of the pups, then crushed bone was substituted which probably prevented tetany during lactation. She remained in apparently good health for forty days after weaning the pups and was used for other experiments.

In another case (A–161) violent tetany developed during lactation with at least four parathyroids intact, as was determined later by operation and autopsy. The regular diet of cooked meat with bread but no bone had been used. A synopsis of the protocol follows.

*Dog A-161.*—Female mongrel; pregnant. Mar. 17, 1909. Thyroid lobes palpable. Sulphur and lard to be given daily (50 gm. of lard containing 5 gm. of powdered sulphur). Weight 9 kilos. Mar. 18. Bit of left lobe, lower pole (weight 0.4 gm.) removed for histological examination. Mar. 21. Stitches removed, wound healed; protective bandage. Mar. 27. Five pups born. Average weight 195 gm. Apr. 2. Weight 7.5 kilos. Stools black and tarry; slight diarrhoea. Apr. 13. Weight 7.2 kilos. Dog is losing weight steadily. This morning a typical and severe tetany developed. At examination the dog is lying on the right side, legs extended, respirations 154. Pulse with stethoscope 224. Marked salivation. Pupils dilated, palpebral fissures also widened. Dilatation of the peripheral vessels. Ears, nose, paws, and skin surface generally hot to the touch. Temperature 104° F. All muscles spastic with fine constant tremors and rapidly recurring coarse jerky twitchings of muscle groups all over the body, in the legs, back, jaws, neck, etc. No obvious gastric dilation. Received one dose of 40 c.c. saturated aqueous solution of calcium chloride by stomach tube, and in twenty minutes she was free from general symptoms of tetany, and able to walk but weak. Sulphur stopped. During the afternoon of the same day another bit of the left lobe, lower pole (weight 1.33 gm.) was removed. The thyroid had enlarged considerably since the last operation. A small IVth parathyroid was attached to the bit of thyroid removed. The dog rallied quickly and ate heartily in the evening. Apr. 14. In excellent condition. Apr. 17. In excellent condition.

Nursing four pups, the other one having been killed at birth as a control. Average weight of the pups is now 755 gm. Apr. 21. Bandage and stitches removed, wound healed, protective bandage. Weight 6.8 kilos. Apr. 22. Started sulphur again, 5 gm. daily. Apr. 29. Definite though mild tetany. Dull but able to stand up, gait ataxic, jaws, legs, back, and head constantly twitching. No tachypnea. Pulse 130. Sulphur stopped but no calcium given or any change in daily routine. Apr. 30. Free from tetany. May 6. Pups weaned. The dog has remained free from any outward signs of tetany, and in the afternoon in attempting operation she choked up with mucus and died in spite of attempts at resuscitation.

*Autopsy.*—Performed at once. Trachea blocked with mucus. Thyroid lobes enlarged. The remaining part of the left lobe weighs 5.22 gm. The intact right lobe weighs 7.92 gm. The two large external or IIId parathyroids are normally placed. The IVth parathyroid on the right lobe is also prominent. No other parathyroid tissue is found. Thymus large and persistent. Spleen normal. Adrenals, liver, kidneys, and lungs, appear normal. No accessory thyroids could be seen or felt in the aortic area.

*Histological Examination.*—The specimen of thyroid removed on Mar. 18 showed colloid early hyperplasia; the specimen removed on Apr. 13 showed colloid early hyperplasia; and the specimen removed on May 6 showed colloid moderate hyperplasia.

The parathyroid cells are large and more distinct than normally. There is no evidence of hyperplasia.

*Summary.*—The bitch developed violent tetany twenty-four days after starting sulphur and seventeen days after the birth of five healthy pups. A single administration of calcium chloride relieved the tetany at once. The sulphur was stopped for nine days following the tetany and then started again. Seven days later the dog developed mild tetany, and on stopping the sulphur no further evidence of tetany developed during the next seven days.

It may be stated, therefore, that in dogs as in man tetanies tend to develop late in pregnancy and occur even more frequently during lactation. The most important question is, Are these pregnancy and lactation tetanies identical fundamentally with those due to parathyroid extirpation? Several authors have answered this question affirmatively. In the cases that I have observed in dogs there are no objective differences to be made out. They present the same clinical manifestations, though usually milder, and they react with calcium salts in the same way.

Since the discovery by MacCallum (14) and Parhon and Ureché (15) of the effect of calcium on parathyroid tetany, it has been suggested that pregnancy and lactation predispose to tetany because at these times the mother suffers an increased depletion of mineral salts among which is calcium. This view draws support from the facts that calcium salts permanently relieve such tetanies, that feeding

foods rich in lime salts or giving lime salts during a second pregnancy will prevent tetany in animals that during their earlier pregnancies developed tetany, and that tetany develops very quickly (six to twelve hours) after removing the parathyroids in lactating or recently lactating animals (Carlson (16)).

I am inclined to think that the administration of large amounts of sulphur favors the development of tetany. From a considerable experience with sulphur in relation to thyroid hyperplasia I came to the conclusion that those dogs which had been given sulphur (and most of our tetany cases had had sulphur) for three to four weeks developed tetany earlier, more violently, and possibly in the presence of more intact parathyroid tissue than otherwise. The case cited above (dog A–161), which cleared up so quickly when sulphur was stopped, reappeared after it was started again, and again cleared up after it was stopped, other conditions remaining constant, was further evidence that the administration of sulphur over long periods of time aids to some extent in bringing about a state of nutrition favoring the development of tetany. How it operates is of course mere speculation. Sulphur is not wholly inert. The formation of sulphides in the intestine and the slight laxative effect suggest that possibly some of the alkaline earths are withdrawn from the field of absorption by this element.

*Influence of Foods on Tetany.*—Meats aggravate the symptoms, and milk, bone, and other foods rich in calcium are protective against tetany or delay its onset. I have seen several instances where bone administration delayed the onset of tetany. No other substance besides calcium has been found to have this effect. Similar effects of an extremely mild and probably more complex nature have been claimed for magnesium and strontium, but in our hands they have yielded negative results. It is certain that tetany is a vastly more complex nutritional disturbance than a calcium deficiency. Of the diseases favoring the development of tetany, rickets stands first. Rachitic pups are more quickly affected by parathyroid removal than normal pups, and tetany is induced following the removal of a smaller amount of parathyroid tissue than in normal pups.[1]

[1] Pups almost invariably become rachitic when fed a diet of bread, liver, lung, and heart muscle, although if crushed bone is added it is prevented. Similar observations have been made with lion cubs at the London Zoölogical Gardens (17).

The close relationship between infantile tetany (carpopedal spasm) and rickets has long been known and many observers believe that this form of tetany is identical with true parathyroid tetany. Yanase (18) reported the finding of hemorrhages in the parathyroids in a significant number of cases of tetany in rachitic infants and believed that a direct relationship existed, but Oppenheimer (19), Bliss (20), Jörgensen (21), and others could not confirm this finding. There could be a grave functional insufficiency without any gross lesions. If the parathyroids were affected we should rather expect an hypertrophy or hyperplasia as a more frequent sign of insufficiency than the presence of atrophic changes, just as occurs in the thyroid. Slight degrees of parathyroid hypertrophy are extremely difficult of proof, as the mammalian parathyroid does not appear capable of a striking overgrowth. Erdheim (22), Bauer (23), and Strada (24) have described hypertrophic changes in the parathyroids in osteomalacia in man. In rickets and infantile tetany it is certain that no marked overgrowth of the parathyroids occurs and calcium does not usually affect this form of tetany. However, that some important connection exists between rickets and infantile tetany and between rickets and the ease and rapidity of onset of true parathyroid tetany in dogs seems established. How much may be due directly to the parathyroids and how much is due to reducing the factors of safety, one of which is certainly calcium, remains unknown.

### RELATION OF THE PARATHYROID GLANDS TO SUGAR TOLERANCE.

Dogs are not favorable animals for this work because it is practically impossible to remove the thyroid lobes without destroying the IVth and seriously injuring the IIId parathyroids. Most of the discordant results reported in the literature are attributable to this factor, a notable illustration being Hirsch's (25) observations, when in 1906 as a result of experiments with six thyroidectomized dogs he concluded that the thyroids exercise an important control over carbohydrate metabolism; and two years later, as a result of experiments on fourteen dogs, he concluded that it was not the thyroid but the parathyroid extirpation that produced glycosuria and lowered

the sugar tolerance. He found the glycosuria and hyperglycemia parallel.

Underhill and Hilditch (26), in 1909, as a result of experiments of thyroparathyroidectomy and partial thyroparathyroidectomy showed that sugar tolerance was measurably reduced when three parathyroids were removed, but that two intact parathyroids could maintain normal control over sugar tolerance. Our observations confirm the above findings in that they show that it is not the loss of the thyroid but of the parathyroids that so quickly lowers sugar tolerance. Blood sugar estimations were not made on any of the cases.

The plan followed was to obtain the average amount of glucose per kilo of body weight that each dog could take without alimentary glycosuria, and then to repeat the observations after parathyroidectomy. The technical routine was as follows: Urine was withdrawn with a catheter, and glucose solution containing 20 gm. of glucose per 100 c.c. of distilled water, to which 0.2 per cent. hydrochloric acid was added, was given by stomach tube. The dogs were catheterized in one and two hours. All dogs were on the regular house diet, fed once daily in the afternoon, and sugar tolerance estimations were made in the morning, an interval of at least three days, and in most cases more, being allowed between observations. The Worm-Müller, Nylander, and Benedict qualitative tests were used with each specimen.

Ten dogs were thus observed and the results are given in table I.

TABLE I.

| Dog No. | Before parathyroidectomy. | | After onset of tetany. | | Presence of sugar in urine unrelated to its administration. | Result. |
|---|---|---|---|---|---|---|
| | No. of observations. | Average tolerance in gm. per kilo. | No. of observations. | Average tolerance in gm. per kilo. | | |
| *A*–167 | 4 | 8 to 9 | | | On 11th day | Fatal. |
| *A*–183 | 4 | 9 to 10 | 8 | 2 to 3 | | Not fatal. |
| *A*–208 | 4 | 8 | 9 | 2 to 3 | Negative | Fatal. |
| *A*–210 | 2 | 7 to 8 | | | On 5th day | Fatal. |
| *A*–213 | 4 | 6 to 7 | 3 | 2 to 3 | Negative | Fatal. |
| *A*–217 | 20 | 2 to 3 | 5 | 2 to 3 | On 3d day | Not fatal. |
| *A*–212 | 5 | 6 to 7 | 1 | 4 | Negative | Not fatal. |
| *A*–214 | 3 | 9 | 3 | 2 | Negative | Not fatal. |
| *A*–226 | 13 | 5 to 6 | 6 | 4 to 5 | Negative | Not fatal. |
| *A*–211 | 6 | 2 to 3 | 21 | 2 to 3 | On 3d day | Not fatal. |

It is seen from the above table that in adult bitches great differences (varying from 9–10 to 2–3 grams per kilo) occur in the natural tolerance from glucose. This must be determined separately, therefore, for each dog. In eight of the ten cases a considerable fall was noticed after parathyroidectomy, while in two with naturally low assimilation limits the tolerance remained unchanged after the removal of at least three parathyroids. The feature of most interest is that parathyroidectomy does not result in constant glycosuria. MacCallum and Voegtlin (27) reported constantly negative results. Such dogs on the average are capable of assimilating from two to three grams per kilo without its appearance in the urine, and more than this quantity is occasionally not tolerated by dogs with intact parathyroids. This suggests that the parathyroids are only a part of a system controlling sugar metabolism. The thyroids were in all these cases slightly goitrous and, for the most part, removed along with the parathyroids, but from data to be published later regarding thyroid and sugar tolerance we have concluded that the presence or the absence of the thyroid has little or nothing to do with this acute change in alimentary sugar tolerance. The administration of calcium salts does not seem to modify the altered sugar tolerance of parathyroidectomy.

### CALCIUM SALTS IN TETANY.

Calcium salts are palliative and preventative, but in no sense curative. MacCallum and Voegtlin (27) have shown that in nearly all cases of parathyroid tetany calcium salts introduced by mouth or intravenously quickly but temporarily relieve the tetany. In this series there was one case where large quantities of calcium chloride by mouth failed to modify the symptoms, and later intravenous injection of considerable quantities likewise failed to modify the symptoms materially. Such cases must be exceedingly rare, however. After total parathyroidectomy calcium salts tend to, and in most cases do, prolong life. The mechanism of its action is unknown. There were several cases in the series that lived 8, 10, 15, and even 20 days with the aid of calcium salts, that as experience has shown usually die in a few hours or a few

days. On the other hand our observations have shown that in cases where a remnant of active parathyroid tissue remains, calcium salts will permanently save such animals which otherwise would succumb to violent tetany. In our series of thirty-eight, four (A–121, A–148, A–183, and A–217) were surely saved by this means. Two others that died of fatal tetany (A–104 and A–115) could in all probability have been saved by the use of calcium salts for a few days as compressed but not necrotic parathyroids were found at the superior pole at autopsy. In tiding over otherwise fatal tetanies, therefore, calcium salts are of the greatest value. This fact is of clinical importance in operations on goitre in man. Total destruction of the parathyroids in the operation for human goitre should not occur except in the most extensive dissections for old complicated goitres and tumors, while partial destruction (the IVth on the given side) normally occurs when lobectomy is performed. The destruction of one or even two under normal conditions in man or dogs produces no untoward effects. When tetanies develop following operations calcium salts are of the greatest value in tiding over acute insufficiencies. The use of calcium is also of value in testing the completeness or incompleteness of the parathyroidectomy. If the operation is complete calcium will not permanently prolong life. As an example of the effect of calcium in prolonging life the following experiment is reported.

*Dog A -121.*—Middle-aged fox terrier; male. Apr. 20, 1908. Weight 7.3 kilos. Following the eleventh thyroid operation on Dec. 22, although 1 c.c. of saturated aqueous solution of calcium chloride was given just after the operation and another 1 c.c. dose on the following morning, he developed mild signs of tetany about noon and in the afternoon the dose was doubled. Dec. 24. Legs spastic, but the animal eats heartily and is able to move about. Weight 11 kilos. Dec. 27. Slight tetany. Dec. 29. Quite marked tetany; very ataxic. 3 c.c. saturated calcium chloride solution. Dec. 31. Slight twitching. Jan. 1, 1909. Well marked tetany; calcium chloride given. Jan. 2. Severe tetany; unable to stand; changed to lactate and given 10 c.c. of 5 per cent. solution daily. Slight signs of tetany each day occasionally reaching severe tetany until Jan. 8; then the dose was increased to 20 c.c. daily. Weight 10 kilos. Eats heartily. Exposure to cold appears to bring on tetany. Jan. 11. Slight tetany. Jan. 14. Marked tetany. Respiration 88. Pulse 180. Abdomen distended. Nose, ears, and feet hot. Jan. 15. A canine parathyroid was transplanted into the right rectus muscle. No calcium was given until Jan. 24, when slight signs of tetany appeared again. Calcium lactate was started again. Jan. 25. Slight tetany. Jan. 26. Violent tetany. Jan. 29.

Violent tetany. Feb. 1. Slight sign of tetany. Feb. 2. Tetany. 10 ox parathyroids given by mouth. Feb. 3. 30 parathyroids given by mouth; definite twitching. Feb. 4. 100 parathyroids given by mouth; twitchings; and in the afternoon tetany became severe and calcium chloride was given. Weight 10.4 kilos. Feb. 6. Slight twitchings relieved by calcium chloride. Feb. 8. Tetany. 50 c.c. of 5 per cent. solution of calcium lactate given. Feb. 9. Under ether one and one half sterile ox parathyroids were inserted into the right rectus muscle, 25 c.c. of calcium lactate being given at the end of the operation. Feb. 10, 11, and 12. Free from tetany. Feb. 13. Tetany. Evidence of gastric irritation, vomits frequently, considerable amounts of mucus. Given 50 c.c. of 0.5 per cent. solution of calcium chloride subcutaneously. Feb. 15. Tetany. Respiration 180. Pulse 210. Apparently suffering pain. Calcium lactate given in milk. Feb. 16. Mild tetany. It appears evident now that calcium salts are not well borne when administered by mouth. Feb. 18. Free from tetany. Calcium lactate given in milk. Feb. 19. The fourteenth operation for thyroid removal; much scar tissue about the remaining stump. The animal rallied quickly, ate heartily, and was given 50 c.c. of 5 per cent. solution of calcium lactate in milk about 6 P. M. Feb. 20, 8 A. M. Dead from hemorrhage.

*Autopsy.*—The hemorrhage is seen to be from the superior thyroid artery which had been cut almost flush with the carotid trunk, and the stump was too short to hold the ligature. Viscera are blanched. Two accessory thyroids, the size of small cherries, were found imbedded in the thymus. Chronic catarrhal gastritis, probably from calcium salts. The other tissues appeared normal. At the time of operation the dog seemed to be in perfect health, and was fat and sleek.

As regards the administration of calcium, the stomach route is preferable to all others and is equally efficient, since the few minutes' quicker action when given intravenously is offset by the greater dangers. Subcutaneous injection is also not to be recommended on account of the irritant action, discomfort, and danger of necrosis. In all our dogs gastric irritation was present. This could have been much lessened by giving the lactate of calcium in milk instead of the highly irritant calcium chloride which is more soluble and acts more quickly. Whether the daily use of calcium as bone would save such dogs is not known. Our few experiences with the temporary use of bone indicate that it also aids.

### PARATHYROID OPOTHERAPY.

Unlike thyroid feeding in myxedema, the feeding of parathyroid glands by mouth has no influence over the course of tetany. MacCallum and Voegtlin (27) reported negative results from their experiments, and we have given as high as 100 fresh ox parathyroids daily to dogs with complete tetany without any amelioration of symptoms or prolongation of life. In another case

(dog A–121) of incomplete but permanent tetany, ten ox parathyroids were given on one day, thirty on the following day, and 100 on the third day, also with negative results. In the same dog one canine parathyroid taken aseptically and inserted into the right rectus muscle maintained the dog free from tetany for eight days, and at the end of this period histological examination of the scar showed no evidence of the parathyroid. A large ox parathyroid was then inserted and this likewise relieved the tetany for five days. MacCallum (27) refers to an observation of Halsted's where feeding ox parathyroids by mouth seemed of value in a case of human tetany, although Shepard (28) reports negative results from a similar experience. MacCallum (29) and others have shown the rapid relief of symptoms by the intravenous injection of fresh parathyroid emulsions, and Beebe (30) has further shown that the parathyroid nucleoproteids also relieve tetany when introduced intravenously.

Transplantation is a difficult procedure and although there are many reports of successes the data of permanency are often incomplete. Halsted (31) points out the necessity of first creating a physiological insufficiency, but even then most of the attempts are failures. Our three attempts to transplant them into the rectus muscle were failures, although the dogs were temporarily relieved of tetany from five to eight days. Bone marrow, spleen, and thyroid are said to be more favorable sites for transplantation.

## SUMMARY.

Accessory parathyroid tissue unassociated with the thyroid lobes is present in 5 to 6 per cent. of dogs. For the determination of the presence of accessory parathyroid tissue there are two tests: (1) the anatomical, often entailing serial sections of the neck and upper thoracic structures, and (2) the biological, consisting of the daily use of some calcium salt for two to three weeks. The latter is more easily carried out and more accurate. In the absence of all parathyroid tissue calcium salts will not save the animal's life, while in the presence of active parathyroid tissue calcium will save it. Many factors other than the amount of parathyroid tissue removed influence the onset of tetany, among which are age, pregnancy, lactation, rachitis, the administration of sulphur, and diet.

Pregnancy and lactation tetany in dogs resembles in all essentials parathyroid tetany. Our observations are in harmony with those who hold that the thyroid and parathyroid are independent structures as regards their anatomy, physiology, and pathology. The removal of the parathyroids immediately lowers alimentary sugar tolerance, but rarely to the degree of constant glycosuria.

In sharp contrast with thyroid substance in myxedema, the feeding of parathyroid substance fresh or dried by mouth is of no value in the parathyroid tetanies of dogs.

Calcium salts have a striking palliative effect on parathyroid tetany and preventative action in tiding over otherwise fatal cases, but are in no sense curative. The mode of action is unknown. There is some evidence that calcium salts directly influence the parathyroid gland.

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# EXPERIMENTAL HYDROCEPHALUS.*

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PLATES 18 TO 21.

Many observations indicate that obstruction to the free communication between the ventricular system of the brain and the subarachnoid space may cause internal hydrocephalus. The obstruction with hydrocephalus has been found either at the foramen of Magendie or in the aqueduct of Sylvius or, in some instances of unilateral hydrocephalus, at the foramen of Monro.

Cases of hydrocephalus following cerebrospinal meningitis in which there was occlusion of the foramen of Magendie have been reported by Quincke (1), Baskett (2), Bramwell (3), and others. Neurath (4) reported a case following scarlet fever in which there was a leptomeningitis with obstruction to the foramen of Magendie. Spiller (5) describes a case of hydrocephalus in which the aqueduct of Sylvius was completely occluded by a proliferation of neuroglia; in the same communication he describes a case of unilateral hydrocephalus in which there was occlusion of the foramen of Monro on the same side by a mass of tissue which he thought was of a tuberculous nature. Schlapp and Gere (6) have reported two cases of internal hydrocephalus in which the aqueduct was closed by proliferation of neuroglia. Barnes (7) reported a case in which there was a tubercle in the roof of the fourth ventricle completely blocking the foramen of Magendie. Merkel (8) and Goldstein (9) have reported cases of hydrocephalus in which blocking of the outflow of fluid was brought about by cysticerci within the aqueduct of Sylvius. Tumors not infrequently cause closure of the aqueduct and are accompanied by hydrocephalus; cases of this kind have been reported by Quincke (1) and Bonhoeffer (10).

Within the past year three cases of brain tumor in which the aqueduct was closed have come to autopsy at the Washington University Hospital. In all of them there was a well marked dilatation of the lateral ventricles.

Although the clinical and pathological aspects of internal hydrocephalus have been the subject of much study, no effort has been made to explain the pathogenesis of the condition by experiment.

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In the literature I found only one instance in which an attempt was made to reproduce the lesion experimentally.

Burr and McCarthy (11) report a case that clinically resembled meningitis, but at autopsy no meningitis was found. There was a moderate degree of internal hydrocephalus with proliferation of the glia and ependymal cells in the lateral ventricles, proliferation of the interstitial tissue in the choroid plexus with partial encapsulation of the plexus, hyaloid bodies, and lymphoid cell infiltration. There was infiltration of the perivascular lymph spaces about the blood vessels beneath the ependyma with lymphoid cells, and dilatation and congestion of these vessels. From the fact that the changes were limited to the ventricles they thought it possible that a toxic substance in the cerebrospinal fluid might have caused the reaction. In order to prove this view they injected toxic substances, such as urine and tuberculin, into the lateral ventricles of kittens. The reaction in the ependyma, the swelling of the glia, and the round cell infiltration of the blood vessels were quite similar to the changes observed in the case described. There was, however, no dilatation of the ventricles.

The following experiments were undertaken to determine if it is possible to cause an obstruction to the outflow of the fluid by plugging the aqueduct or foramen of Magendie and to produce hydrocephalus by injecting some insoluble substance into the ventricle. Aleuronat was chosen for this purpose; it is a finely granular substance insoluble in the body fluids and produces a well marked inflammatory reaction. Dogs were used in all the experiments.

### TECHNIQUE.

The dog was etherized, the head shaved, and a longitudinal incision was made in the median line over the parietal bones. The temporal muscle was cut across near its origin and separated from the surface of the bone. A small button of bone five millimeters in diameter was removed with a trephine at a point about one centimeter behind a line drawn over the vertex of the skull joining the anterior margins of the ears and about two centimeters from the median line. On account of the difference in size of the animals and the shape of the heads it is impossible to lay down a hard and fast rule by which this point may be found. The hemorrhage if excessive was controlled with bone wax. The dura was punctured with a sharp needle or with the point of a knife. For puncturing the ventricle a blunt needle with a lateral opening near the end was used. This needle was inserted slowly in a downward and inward direction

until penetration into the ventricle was indicated by the flow of cerebrospinal fluid. The depth to which the needle must be inserted in order to reach the ventricle varies, of course, with the size of the dog, being as a rule between one and a half and two centimeters. After a few drops of cerebrospinal fluid were allowed to escape, the aleuronat suspension[1] was injected by means of a small Luer syringe connected directly with the needle while it lay in position with its tip in the ventricular cavity. The needle was withdrawn, and the muscle and skin were closed with silk. At the death of the animal the brain was hardened *in situ* by injecting 10 per cent. formalin into the carotid arteries.

For purposes of description the experiments may be divided into groups depending on the length of time the animals lived after the operation. The experiments are described briefly to show the relation between symptoms during life, the gross changes in the ventricles of the brain, and the stage of the inflammatory process affecting the ependyma.[2]

GROUP I. EXPERIMENTS LASTING LESS THAN ONE WEEK.

*Dog 6.*—2 c.c. of the aleuronat suspension were injected into the right lateral ventricle. The animal appeared sick the next day, seemed to be in pain, and was unable to stand. On the following day there was some improvement, but it was still unable to stand. It was killed with chloroform at the end of the third day.

*Gross Examination.*—The aleuronat suspension was found in both lateral ventricles; the third and fourth ventricles and the aqueduct of Sylvius were plugged with the aleuronat. There was a small hemorrhage in the optic thalamus on the right side and some blood in the right lateral ventricle. This hemorrhage was undoubtedly due to the insertion of the needle beyond the ventricle. There was no dilatation of the ventricles.

*Microscopic Examination.*—There is an abundance of aleuronat surrounded by polynuclear leucocytes in both lateral, the third and fourth ventricles, and in the aqueduct of Sylvius. The choroid plexus is edematous and is infiltrated

[1] The aleuronat was suspended in starch. It was prepared as follows:

| | |
|---|---|
| Aleuronat | 5.0 |
| Starch | 1.5 |
| Water | 75.0 |

The starch was placed in cold water; this was slowly brought to the boiling point and the aleuronat added and thoroughly mixed. The mixture was sterilized in the autoclave.

[2] I am under obligation to Dr. Ernest Sachs for the ophthalmoscopic examinations.

with immense numbers of polynuclear leucocytes. There is some proliferation of the ependymal cells lining the ventricular cavities and a slight swelling of the neuroglia beneath the ependyma. The walls of the blood vessels situated beneath the ependyma and for some distance in the brain substance are infiltrated with polynuclear leucocytes.

*Dog 14.*—0.75 c.c. of the aleuronat suspension was injected into the right lateral ventricle. The dog appeared well until the third day when the head was seen to be hyperextended and the legs spastic. Death occurred on the fifth day.

*Gross Examination.*—There was a small hernia of cerebral tissue through the opening in the dura, which extended to the outer surface of the skull. There was hemorrhage and exudate at the base of the brain and about the medulla and extending into the spinal canal. On section the lateral ventricles were slightly dilated. The foramen of Monro on the right side was closed with aleuronat. Both lateral, and the third and the fourth ventricles were filled with the aleuronat. The aqueduct of Sylvius was clear.

*Microscopic Examination.*—All the ventricles contain a large amount of aleuronat surrounded by polynuclear leucocytes. The ependymal cells are proliferated, and there is an accumulation of lymphoid cells beneath the ependyma; the walls of the blood vessels show the same infiltration with polynuclear leucocytes. In the pia at the base of the brain and on the surface of the pons, medulla, and cord there are large numbers of polynuclear leucocytes; there is a small bit of the aleuronat on the posterior surface of the medulla.

*Dog 26.*—1 c.c. of the aleuronat was injected into the right lateral ventricle. On the second day the dog appeared quite ill; pressure over the scalp was obviously painful. On the third day the animal was unable to stand and there was a paralysis of the left side of the body. On the fourth day there was a definite cellulitis of the scalp. The animal was killed with chloroform at the end of the fourth day.

*Gross Examination.*—On section both lateral ventricles were slightly dilated; they measured 13 by 10 mm. at the level of the infundibulum. Aleuronat could be seen in the right lateral and the third ventricles. The aqueduct was patent.

*Microscopic Examination.*—The right lateral ventricle contains some blood and many polynuclear cells. Polynuclears are seen in the choroid plexus in all the ventricles, and there are a few large mononuclear cells. Small bits of the aleuronat are present in all the ventricles, more in the right lateral ventricle than elsewhere.

In these experiments there is very slight dilatation of the ventricles. In each instance the exudate is composed almost entirely of polynuclear cells. The walls of the blood vessels are infiltrated with the same cells. In dog 14 there was an acute meningitis probably due to the irritation caused by aleuronat which had found its way into the subarachnoid space through the foramen of Magendie, though possibly by an infection. The picture is one of an acute inflammation.

The experiments show that aleuronat injected into the right ven-

tricle finds its way into the other ventricles. Masses of the substance may plug the aqueduct of Sylvius (dog 6) or the foramen of Monro (dog 14).

GROUP II. EXPERIMENTS ENDING BETWEEN THE SEVENTH AND FOURTEENTH DAYS.

*Dogs 21, 22, and 24.*—Puppies of the same litter one week old. The needle was inserted at the usual point in the direction of the ventricle and 0.25 c.c. of the aleuronat suspension was injected. In none of the experiments was it certain that the ventricle had been punctured. All the puppies appeared normal after the operation. No. 21 died ten days after the injection. Nos. 22 and 24 died on the twelfth day. Dog 23, which belonged to the same litter, died thirty-eight days after the injection and is described in group IV. Since the changes in Nos. 21, 22, and 24 were practically the same, one description will answer for all.

*Gross Examination.*—Considerable dilatation of the ventricles; at the level of the optic commissure the lateral ventricle varied from 6 by 8 mm. to 10 by 10 mm. on the right side, and on the left from 3 by 4 to 5 by 7 mm. The aleuronat was seen in both lateral ventricles. The foramen of Monro on the right side where dilatation of the ventricle is greater in each instance was plugged with aleuronat; the aqueduct of Sylvius was patent throughout.

*Microscopic Examination.*—The choroid plexus in the lateral ventricle (more marked in the right) is covered with aleuronat and an exudate composed of immense numbers of small and large mononuclear cells; there is proliferation of connective tissue cells from the choroid, and here and there a giant cell. The cells of the ependyma are proliferated and in many places form several layers; they have a tendency to assume a columnar shape instead of the normal low cuboidal cell. The walls of the blood vessels beneath the ependyma are infiltrated with small mononuclear cells. The choroid plexus in the fourth ventricle shows some proliferation of the ependymal cells and a moderate degree of infiltration with lymphoid and polynuclear cells.

In these experiments the dilatation of the ventricles is distinct. The inflammation is more advanced than in group I; there are lymphoid and large mononuclear cells and some proliferation of the connective tissue cells in the choroid plexus; polynuclear cells are still present in large numbers.

GROUP III. EXPERIMENTS ENDING BETWEEN THE FOURTEENTH AND TWENTY-EIGHTH DAYS.

*Dog 2.*—2 c.c. of the aleuronat suspension were injected into the left lateral ventricle. The dog was killed on the nineteenth day and the brain hardened *in situ*.

*Gross Examination.*—On section there was definite dilatation of both lateral

ventricles, the third, and to a slight extent the fourth. The lateral ventricles at the point of greatest dilatation measured 12 by 6 mm. A small bit of the aleuronat was seen in the descending horn of the left lateral ventricle.

*Microscopic Examination.*—The floor of the third ventricle shows a proliferation of the glia and an accumulation of lymphoid cells, plasma cells, large mononuclear cells, and giant cells. In the aqueduct there is a small bit of the aleuronat surrounded by the same cells, the proliferation of ependyma is well marked. The choroid plexuses in the lateral ventricles are infiltrated with lymphoid cells and there is proliferation of the connective tissue; that in the fourth ventricle appears normal.

*Dog 9.*—1 c.c. of the aleuronat suspension was injected into the right lateral ventricle. The animal behaved normally until the tenth day when it became inactive and looked sick; the hind legs were held stiffly in walking, and manipulation of the legs was met with resistance suggestive of muscle spasm; this resistance to motion had increased somewhat on the fifteenth day, and then remained about the same until death on the twentieth day. Evidence that the brain tissue was under pressure was afforded by a hernial protrusion of the cerebral tissue through the trephine opening. The cerebellum, moreover, projected through the foramen magnum and there was a well defined groove at the point where the bony margin of the foramen was in contact with the cerebellar tissue. On section of the brain at the level of the optic commissure there was a well defined dilatation of the lateral ventricles, the right measuring 6 by 10 mm., the left 10 by 15 mm.; the third and fourth ventricles were not dilated and the aqueduct of Sylvius was patent. No exudate could be seen on gross examination. The ophthalmoscopic examination made shortly before death showed a choked disc on both sides. Figure 1 is from a normal brain for comparison; figure 2 is from the brain of dog 9.

*Microscopic Examination.*—There is some proliferation of the ependymal cells lining the ventricles; the choroid plexus (figure 10) in the lateral and third ventricles has aleuronat imbedded in its folds and surrounded by small and large mononuclear cells, connective tissue cells, and a few giant cells. In the fourth ventricle there are a few small mononuclear and polynuclear cells. There is marked proliferation of the ependymal cells lining the aqueduct of Sylvius (figure 11), and the blood vessels under the ependyma show the same infiltration with small mononuclear cells as that described above.

*Dog 10.*—1 c.c. of the aleuronat suspension was injected into the right lateral ventricle. There was at first some stiffness of the legs and on the sixteenth day there was what appeared to be spastic paraplegia. The eyes were clear but the animal appeared to be blind. It died on the eighteenth day.

*Gross Examination.*—At the site of puncture the cerebral tissue protruded through the trephine opening for a distance of about four millimeters. The cerebellum shows a groove corresponding to the edge of the foramen magnum. On section both lateral ventricles were dilated, the left measuring 8 by 10 mm., the right 5 by 7 mm. The right foramen of Monro was plugged with aleuronat. There was no dilatation of the third ventricle. The fourth ventricle was compressed. The aqueduct of Sylvius was closed.

*Microscopic Examination.*—The choroid plexus in the right lateral ventricle is distorted and thickened; there is a mass composed of connective tissue cells,

large mononuclear cells, lymphoid cells, and some giant cells. In the left lateral ventricle the choroid plexus shows no change except slight edema. In the walls of the right ventricle the walls of the blood vessels are infiltrated with lymphoid cells in considerable numbers (figure 9); this change is not seen to the same extent on the left side. The aqueduct of Sylvius is completely plugged with aleuronat, necrotic tissue, lymphoid cells, and connective tissue cells.

*Dog 16.*—1 c.c. of the aleuronat suspension was injected into the right lateral ventricle. The dog remained well until the twentieth day when it appeared ill; it gradually grew worse and died on the twenty-fifth day.

*Gross Examination.*—Both lateral ventricles were dilated (figure 3); they measured about 10 mm. in diameter at the level of the hypophysis. The third ventricle, the anterior part of the aqueduct of Sylvius, and the fourth ventricle were slightly dilated. The aqueduct was patent throughout. The foramina of Monro appeared to be open.

*Microscopic Examination.*—The choroid plexus in the lateral ventricle is thickened. There is infiltration of lymphoid, large mononuclear, and connective tissue cells. The walls of the blood vessels are infiltrated with lymphoid cells.

*Dog 17.*—0.75 c.c. of aleuronat suspension was injected into the right lateral ventricle. The animal remained well for two weeks, then began to lose weight, became inactive and gradually sank into a comatose condition. Death occurred on the twenty-third day after operation.

*Gross Examination.*—Both lateral ventricles were dilated (figure 4), the right measuring 6 by 4 mm., the left 9 by 10 mm. The aqueduct of Sylvius was patent and apparently slightly dilated; the third ventricle was not dilated, nor was the fourth. The foramen of Monro on the right side was occluded.

*Microscopic Examination.*—The choroid plexus of the lateral ventricle is thickened and infiltrated with small and large mononuclear cells and a few giant cells; there is marked proliferation of the glia beneath the ependyma (figure 12) and of the ependymal cells. No polynuclear cells are seen except in the fourth ventricle where there are a few in the choroid plexus.

In this group of experiments the signs of acute inflammation have entirely disappeared and there is more marked increase of connective tissue in the chloroid plexus; plasma cells occur in considerable number. There is a greater proliferation of ependyma.

The animals remained well during the period from ten to twenty days and displayed symptoms suggesting cerebral pressure. In one instance the occurrence of choked disc was demonstrated. Further evidence of increased intracerebral pressure was furnished by hernial protrusion of the brain substance through the trephine wound and by projection of the cerebellum into the foramen magnum. These changes were explained by well marked internal hydrocephalus found in this group of experiments.

GROUP IV. EXPERIMENTS OF DURATION LONGER THAN TWENTY-EIGHT DAYS.

*Dog 19.*—Puppy five weeks old. In puncturing the brain no cerebrospinal fluid was obtainable. 0.5 c.c. of the aleuronat suspension was injected with the needle in the position at which the ventricle was usually found. The dog recovered readily and appeared to be well. On the thirtieth day a second attempt was made. Again it was impossible to obtain cerebrospinal fluid. 0.5 c.c. of the aleuronat was injected. Sixteen days later a third puncture was made, 1 c.c. of cerebrospinal fluid was removed and 0.25 c.c. of aleuronat injected. On the following day the dog was very restless; on the next day he was unable to stand, the eyes were staring, and the head was jerked to the right at short intervals. Both lateral ventricles were tapped, and 1.5 c.c. of fluid were withdrawn from each. Fluid from the right was turbid and contained many polynuclear cells. Fluid from the left was clear and colorless. The next day the animal was much better and was able to walk, the eyes remained staring, and the upper lip contracted spasmodically. Examination of the eyes showed well marked choked disc on both sides. The animal was killed with chloroform thirty days after the last puncture and three months and seven days after the first operation.

*Gross Examination.*—Both lateral ventricles were dilated. The anterior horn of the right lateral ventricle was more widely dilated than that of the left and measured 12 cm. in diameter, while the posterior and descending horns of the left were greater than the right, the greatest diameter being 20 mm. The anterior horn of the right ventricle was divided by thin friable septa into several small compartments filled with a semifluid opaque material. The left ventricle did not have these septa and was filled with colorless fluid. The third ventricle was not dilated.

*Microscopic Examination.*—The choroid plexus on the right side is thickened with connective tissue and there is some proliferation of the ependymal cells. There is some granular detritus in the folds of the choroid plexus and many large mononuclear cells containing a light brown pigment. The ependymal lining of the ventricles consists of a single layer of flattened cells. In the choroid plexus there are a great many large mononuclear cells containing brown pigment granules.

*Dog 20.*—Puppy five weeks old from the same litter as No. 19. On puncture cerebrospinal fluid was not obtained. 0.5 c.c. of the aleuronat suspension was injected into the right side. The animal appeared quite well following the operation. On the thirtieth day after the first operation the ventricle was punctured, some of the fluid withdrawn, and 0.25 c.c. of aleuronat was injected. Seventeen days later 0.25 c.c. was injected into the right ventricle. On the following day the hind legs were rigid, the dog was unable to stand, and the eyes were staring, exhibiting some exophthalmus. Death on the second day following the last operation was fifty-two days after the first.

*Gross Examination.*—There was a small cerebral hernia at the site of puncture. On section the ventricles were greatly dilated (figure 5), the right measuring 8 by 10 mm. at the level of the optic commissure, the left 13 by 18 mm. The greatest dilatation was seen in the descending horns, the left measuring 10 by 13 mm., the right 18 by 13 mm. In the right ventricle there were soft thin

septa dividing the ventricle into several compartments. The third and fourth ventricles were not dilated. The aqueduct of Sylvius was patent throughout.

*Microscopic Examination.*—The right lateral ventricle contains a large quantity of exudate made up chiefly of polynuclear cells. This acute inflammation seems to be superimposed on one of a more chronic character in which the choroid plexus is thickened by connective tissue cells infiltrated with lymphoid and large mononuclear cells. The blood vessels in the walls of the right ventricle are infiltrated with lymphoid cells, large mononuclear cells, and in places with polynuclears. The choroid plexus in the third and left lateral ventricles appears normal; that in the right lateral ventricle is thickened by connective tissue. The ependymal cells have practically disappeared and there are a great many polynuclear cells in the plexus and infiltrating the walls of the blood vessels in the walls of the ventricles. The proliferation of ependyma is well shown in the aqueduct of Sylvius where there is also an apparent increase in neuroglia. The choroid plexus in the fourth ventricle is somewhat edematous.

*Dog 23.*—Puppy from the same litter as group II. The animal died thirty-eight days after operation.

*Gross Examination.*—Both ventricles were dilated, the right slightly more than the left; it measures 8 by 10 mm. at the level of the optic commissure. In the posterior horn of the right lateral ventricle there was a cavity in the brain substance 12 mm. in diameter, extending to within 2 mm. of the cortex; this was connected with the ventricle by an opening about 7 mm. in diameter. It was presumably the site of puncture and the aleuronat, doubtless injected into the brain substance, by destruction of the brain tissue gained entrance to the ventricle. The cavity contained a small amount of semifluid pus-like material. The third and fourth ventricles were not dilated. The aqueduct of Sylvius was patent.

*Microscopic Examination.*—A section through the wall of the cavity shows the picture of brain softening; there is a loose network containing degenerated cells and many compound granular corpuscles, some of which contain brown pigment granules. The wall of the ventricle is infiltrated with lymphoid cells. The choroid plexus is infiltrated with lymphoid and large mononuclear cells and in places with small hemorrhages surrounded by polynuclear cells.

*Dog 38.*—Puppy two days old. A few drops of aleuronat were injected into the right hemisphere in the region of the ventricle. Death occurred sixty days after the operation.

*Gross Examination.*—The right ventricle showed enormous dilatation (figure 6), measuring 18 by 20 mm. in diameter at the level of the hypophysis. The left ventricle showed no dilatation. The aqueduct of Sylvius was patent. There was no dilatation of the third or fourth ventricles.

*Microscopic Examination.*—A section of the wall of the right ventricle shows slight infiltration with lymphoid cells and the neuroglia is somewhat increased. There is no proliferation of the ependyma except in the aqueduct.

All the dogs in this group were very young puppies. In none of them was it certain at the time of operation that the ventricle was punctured. In this group the animals lived more than four weeks and the dilatation of the ventricles is considerable. The

microscopic changes differ very little from those found in the preceding group. In No. 20 there is an acute inflammation following the last injection which was made only two days before death.

In the experiments of group I in one instance (dog 6) the aqueduct of Sylvius, and in another the foramen of Monro was plugged with aleuronat. In group II in every instance with well marked dilatation the right foramen of Monro was plugged. In group III in dog 10 the aqueduct of Sylvius was completely plugged with aleuronat and newly formed tissue and there was well marked dilatation of the ventricles. In dog 17 the foramen of Monro on the right side was plugged. It has not been possible to determine with certainty the condition of the foramen of Magendie, but proliferation of tissue in the roof of the fourth ventricle and elsewhere has suggested the possibility of occlusion by newly formed tissues. A more accurate method of determining the patency of this opening was desirable.

It was thought that the injection of a pigment into the ventricles of dogs with hydrocephalus would make it possible to determine if fluid no longer passed through the foramen of Magendie. For this purpose black India ink was used in a dilution of one part to four parts of water. The following experiment was done on normal animals.

One cubic centimeter of the suspension of the India ink was injected under ether into the left lateral ventricle of three normal dogs (Nos. 32, 33, and 34). They were killed with chloroform at the end of one, one half, and one quarter hours, and the brains hardened *in situ* by formalin injected into the carotid artery. On examination the ink, fixed in position by the formalin, was found in large quantities in the pia arachnoid at the base of the brain, being more abundant around the base of the cerebellum in the region of the foramen of Magendie than elsewhere, but in all three brains the pigment was seen extending over the cerebral hemispheres. There were well defined differences dependent upon the duration of the experiment. Pigmentation was most intense in the dog that had been allowed to live one hour, slightly less in the one living one half hour, and less, but still very distinct, in the third living only one quarter hour after the injection. In the third animal there was

almost no pigment over the upper half of the cerebral hemispheres. The pigment was confined to the subarachnoid space, and on tearing away the pia arachnoid the brain was left quite clean. On section the pigment was seen distributed throughout the ventricular system in all the brains. On microscopic examination the pigment in the ventricles was attached to the ependymal cells, and in no place was it found beneath the ependyma. The experiment offers a method of demonstrating the gradual flow of cerebrospinal fluid from the ventricles of the brain into the subarachnoid space through the foramen of Magendie.

In the following instances the same experiment was carried out on dogs in which hydrocephalus had previously been produced by injection of aleuronat.

*Dog 8.*—1.5 c.c. of the aleuronat suspension were injected into the right lateral ventricle. The animal recovered readily and remained well for four months. Under ether the left side of the skull was trephined and after drawing off about 1 c.c. of cerebrospinal fluid 1 c.c. of the suspension of India ink was slowly injected. One half hour later the dog was killed.

*Gross Examination.*—The surface of the brain is clear and white, none of the ink being visible. On section the lateral ventricles are dilated equally (figure 7) and measure about 12 mm. in diameter. The left lateral ventricle, the third and fourth ventricles, and the aqueduct of Sylvius contain the ink, but there is none in the right lateral ventricle. The foramen of Magendie is readily made out but seems to be closed by a thin web-like membrane.

*Microscopic Examination.*—In sections through the fourth ventricle at the site of the foramen of Magendie there is marked proliferation of ependyma on the floor of the ventricle and a fine network of neuroglia covering the ependyma and containing within the meshes some large mononuclear cells. The roof of the ventricle is composed of choroid plexus arising from each side of the pons and joined in the center by thin strands of connective tissue containing a few small blood vessels. The plexus itself appears edematous, but otherwise is normal. These changes occur at the level of the foramen of Magendie which is apparently closed by the connective tissue.

*Dog 11.*—1 c.c. of the aleuronat suspension was injected into the right lateral ventricle. The dog lived for four months and eight days and was then killed with chloroform, one half hour after injecting 1 c.c. of the diluted India ink into the left lateral ventricle.

*Gross Examination.*—There was a small amount of the ink in the pia at the base of the brain, but much less than that found fifteen minutes after injection of ink into the ventricle of the normal dog already mentioned. There was no evidence of great pressure, no cerebral hernia, nor jamming of the cerebellum into the foramen magnum. On section both lateral ventricles were dilated equally, measuring about 12 by 15 mm. at the level of the hypophysis. In the

posterior horn of the right lateral ventricle was a thin septum of friable tissues. The left lateral ventricle and the third ventricle were filled with ink. There was none in the right ventricle. The anterior end of the aqueduct of Sylvius was very much narrowed, while the posterior end was dilated. The roof of the fourth ventricle was accidentally torn in the examination, making it impossible to determine whether it was closed or not.

*Microscopic Examination.*—There is some proliferation of the neuroglia in the right ventricle. The ventricles are lined by a single layer of flattened cells. In the left ventricle the pigment is seen attached to the lining cells. In the aqueduct of Sylvius there is proliferation of the ependyma and occasionally a small nodule covered by ependyma resembling closely those seen in granular ependymitis.

*Dog 25.*—1 c.c. of aleuronat suspension was injected into the right lateral ventricle. Sixty-eight days after the operation 1 c.c. of India ink was injected into the left lateral ventricle and one half hour later the dog was killed with chloroform.

*Gross Examination.*—There was no ink on the surface of the brain and none at the base. On section both lateral ventricles were dilated; the left measured 10 by 10 mm. at the level of the infundibulum, and the right at the same level was much larger, measuring 22 by 15 mm. The dilatation was more marked in the descending horn but was considerable throughout. The ink was found in the left lateral, the third, and the fourth ventricles, but none was present in the right. The aqueduct of Sylvius was narrowed but patent. The foramen of Magendie was closed by a very thin membrane.

*Microscopic Examination.*—The lateral ventricles are lined by a single layer of flattened ependymal cells. There is some increase in the neuroglia and occasionally there is a small granulation similar to those seen in the aqueduct of Sylvius in the preceding case. In the aqueduct of Sylvius there is some increase in the neuroglia and proliferation of ependyma. The India ink is readily seen in the aqueduct and fourth ventricles. A section through the roof of the fourth ventricle shows that the foramen of Magendie is closed by a thin connective tissue membrane similar to that found in the preceding experiment.

*Dog 37.*—No cerebrospinal fluid could be obtained on puncture, but 0.75 c.c. of the aleuronat was injected into the left cerebral hemisphere with the needle held in the position at which the ventricle was usually found. Sixty-six days afterwards 1 c.c. of the India ink was injected into the left lateral ventricle and a half hour later the animal was killed.

*Gross Examination.*—A small amount of the ink was found at the base of the brain, but there was none on the surface of the cerebrum or cerebellum. On section both lateral ventricles were found moderately dilated. The anterior horns were but little affected and the body of the ventricles at the level of the posterior edge of the hypophysis measured 8 by 8 mm. The posterior horns were moderately dilated. The left lateral ventricle was filled with the ink and there was a very small bit in the right lateral ventricle and in the third and fourth ventricles. A section through the optic thalamus shows a small yellow area in its center.

*Microscopic Examination.*—The ependyma of the left ventricle shows proliferation only at a point where the choroid plexus is attached, and here large

mononuclear cells bearing yellow pigment are seen in the choroid plexus. The lining of the ventricle is covered with the ink. In the optic thalamus is an area in which the brain substance is destroyed and replaced by compound granular cells embedded in a fine network. This is undoubtedly the site of an injury caused by the needle. A section through the wall of the right ventricle shows no abnormality.

*Dog 42.*—0.75 c.c. of the aleuronat was injected into the right lateral ventricle; forty-seven days later 1 c.c. of the India ink was injected into the left lateral ventricle, and one half hour later the animal was killed.

*Gross Examination.*—The entire surface of the brain was clean, no trace of the ink being seen. On section the ink was found only in the ventricle injected (figure 8). The right lateral ventricle measured 15 mm. in diameter at the level of the infundibulum, while the right measured 10 mm. The descending horns were more widely dilated, the right measuring 20 mm. from above downward.

*Microscopic Examination.*—The right ventricle is lined by a loose network of neuroglia which is apparently edematous; the ependyma occurs as a single layer of flattened cells. The choroid plexus is thin in places and has the appearance of being stretched out; in one area there is nothing but a long double line of flattened cells, and in another area there is merely a line of connective tissue in contact with a dilated vein. The aqueduct of Sylvius is almost obliterated by neuroglia and the ependyma shows marked proliferation.

The experiments with India ink show that obstruction may occur at the foramen of Magendie and at the foramen of Monro. The third and fourth ventricles, surrounded perhaps by more resistant tissue, may exhibit no dilatation. The same condition is found in hydrocephalus in man where the dilatation was brought about by obstruction in the fourth ventricle or the aqueduct of Sylvius; the third ventricle may or may not be dilated.

## SUMMARY.

Internal hydrocephalus can be produced experimentally by injecting a foreign substance into the ventricles. In these experiments, aleuronat, a granular, insoluble material, has caused an acute inflammatory reaction, characterized in the first week by an exudate consisting largely of polynuclear leucocytes. Later the picture is one of a chronic process; polynuclear cells are replaced by lymphoid and large mononuclear cells and there is proliferation of the connective tissue in the choroid plexus. Proliferation of the ependyma occurs in the first week but becomes more advanced in the second and third weeks, and there is increase in neuroglia more marked in the long continued experiments.

Ordinarily when aleuronat is injected into a serous cavity, the pleural cavity, for example, abundant accumulation of fluid takes place in twenty-four to forty-eight hours, and at the same time polynuclear leucocytes collect. In the experiments in which the irritant was injected into the ventricle of the brain there was little or no dilatation apparent in the first week; absence of dilatation in all probability is due to the free outflow of the fluid. When obstruction occurs during the chronic stage of the inflammatory process dilatation of the ventricle follows. Choked disc and other symptoms of increased intracranial pressure accompany experimental hydrocephalus.

Dilatation occurs slowly and reaches a maximum in about two months. In some of the experiments of longer duration obstruction can be demonstrated in gross or microscopically. Obvious obstruction has not been found by gross examination in all instances, but in the experiments in which India ink was injected into the ventricle before death obstruction to outflow was very readily demonstrated. The third and fourth ventricles were in all instances filled with the pigment, but none appeared on the surface of the brain, whereas in normal dogs the entire surface, especially the base, became deeply pigmented. Obstruction to the circulation of cerebrospinal fluid causing internal hydrocephalus may occur at the foramen of Monro, in the aqueduct of Sylvius, or, doubtless with greatest frequency, at the foramen of Magendie.

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## EXPLANATION OF PLATES.

### PLATE 18.

FIG. 1. Normal brain.

FIG. 2. Dog 9, group III, showing dilatation of the lateral ventricles.

FIGS. 3 and 4. Dogs 16 and 17, group III, showing dilatation of the lateral ventricles.

### PLATE 19.

FIG. 5. Dog 20, group IV, showing marked dilatation of the lateral ventricles with the septa dividing the right.

FIG. 6. Dog 38, group IV, showing dilatation of the right lateral ventricle.

FIG. 7. Dog 8, showing dilatation of both lateral ventricles and the presence of the India ink in the left lateral and the third ventricle and in the aqueduct of Sylvius.

FIG. 8. Dog 42, showing dilatation of the lateral ventricles and the India ink in the left lateral. The photograph does not show the ink in the third ventricle and the aqueduct of Sylvius; this was plainly seen in the specimen.

### PLATE 20.

FIG. 9. Dog 10, group II, showing the accumulation of lymphoid cells about the blood vessels in the ventricular walls.

FIG. 10. Dog 9, group III, showing thickened choroid plexus.

### PLATE 21.

FIG. 11. Dog 9, group III, showing proliferation of the ependyma in the aqueduct of Sylvius.

FIG. 12. Dog 17, group III, showing proliferation of neuroglia in the wall of the lateral ventricle.

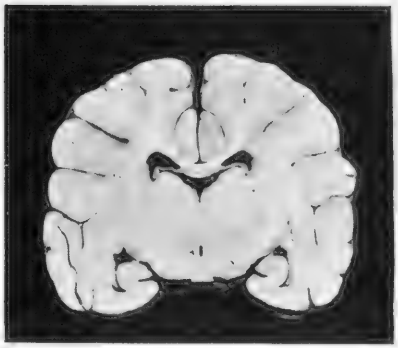


FIG. 1.

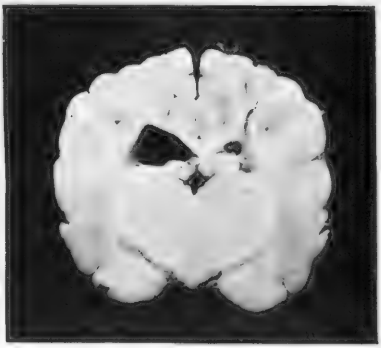


FIG. 2.

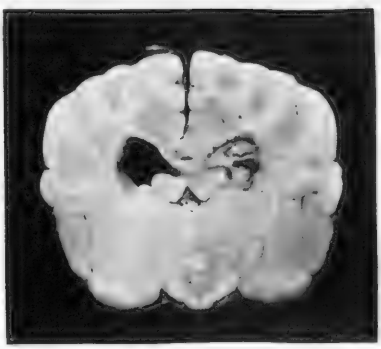


FIG. 3.

FIG. 4.

(Thomas: Experimental Hydrocephalus.

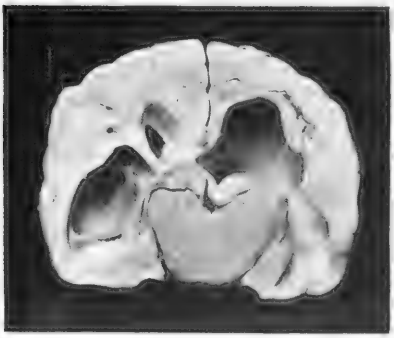


FIG. 5.

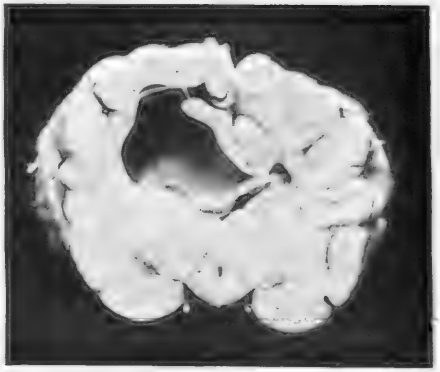


FIG. 6.

FIG. 7

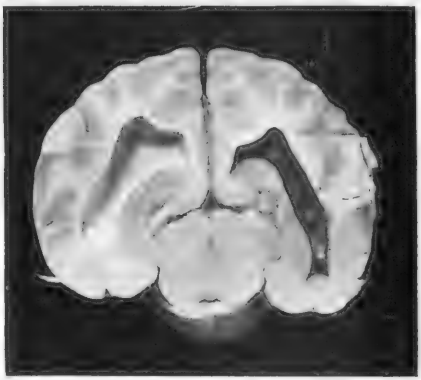


FIG. 8.

(Thomas: Experimental Hydrocephalus.)

U of M

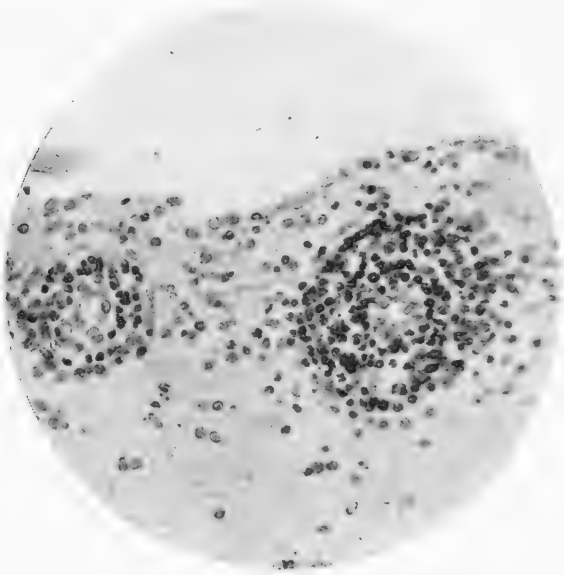


FIG. 9.

FIG. 10.

(Thomas: Experimental Hydrocephalus.)

U of M

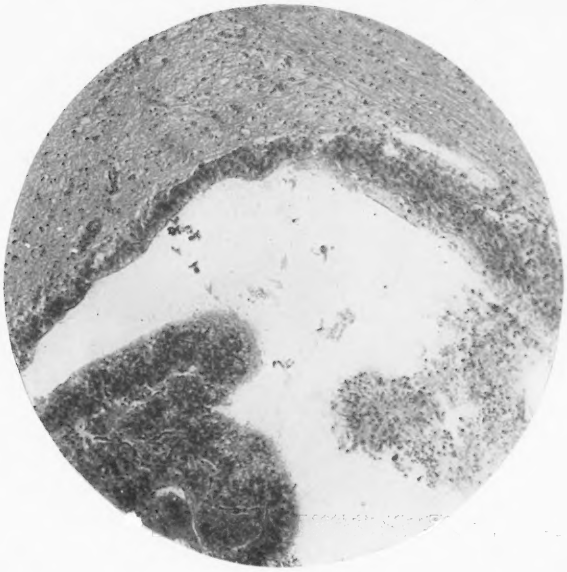
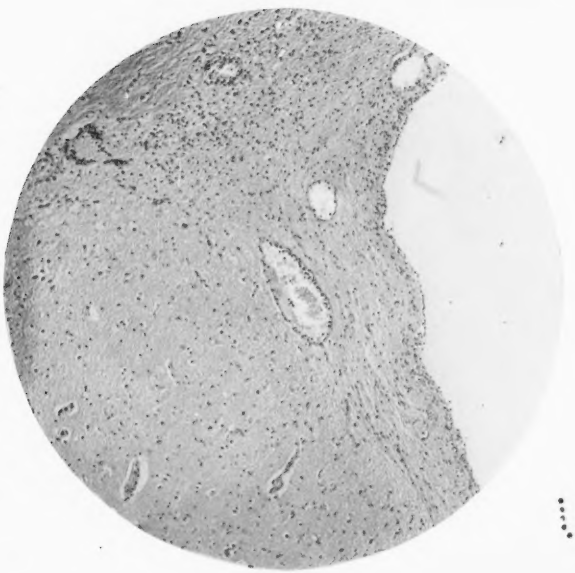


FIG. 11



UofM

FIG. 12.

(Thomas: Experimental Hydrocephalus.)

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